Continuous-flow cryocompression therapy after hip fracture surgery

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Contents

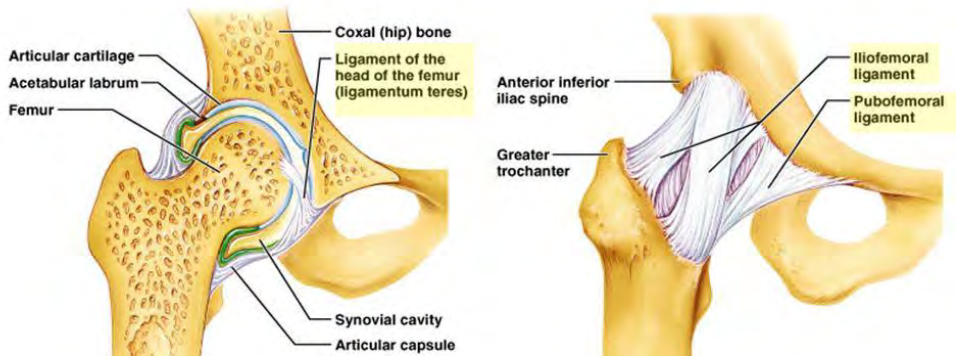
Chapter 1

General introduction and outline

Hip anatomy

The hip joint is one of the most important weight-bearing ball and socket synovial joints of the human body. It enables us to execute movements such as walking and running, but also allows us to remain balanced while in a sedentary position. The hip joint links the pelvis with the femur, thereby connecting the axial skeleton with the lower extremity where it bears the weight of our body, and conducts the forces of the surrounding muscles (Figure 1). Yet the hip joint is also one of our most flexible joints, and allows a greater range of motion than all other joints in the body except for the shoulder. Fibrous ligaments attach proximally to the acetabulum, and insert distally to the neck of the femur and at the greater trochanter, hereby forming strong capsular connection between the ball (femoral collum) and the socket (acetabulum; Figure 1). The arterial supply to the hip joint is largely via the medial and lateral circumflex femoral arteries, which are branches of the profound femoral artery. These arteries anastomose at the base of the femoral neck to form a ring, from which smaller arteries arise to supply the hip joint itself, and the artery to head of the femur and the gluteal arteries provide some additional supply. The medial circumflex femoral artery is responsible for the majority of the arterial supply. Damage to the medial circumflex femoral artery, for instance in the case of a fracture, can result in delayed fracture healing or even in avascular necrosis of the femoral head.

Figure 1. Normal bone anatomy of the hip joint with surrounding ligaments.



Fracture of the hip

Proximal femur fractures or hip fractures are among the most common injuries in trauma[1]. The last decades global hip fracture incidence is steadily rising, and figures are projected to increase from 1.6 million in 2000[2] to more than 6 million in the year 2050[3–6]. Within The

Netherlands 7,614 patients were treated for a hip fracture in 1981, while in 2010 this figure has risen to 21,000 on an annual basis[7]. Hip fractures are strongly related to reduced bone mineral density and falls are responsible for at least 90% of all hip fractures[8]. After the menopause in women and with advancing age in men, bone mineral density reduces and neuromuscular function declines, these changes together produce a rapid rise in fracture risk (Figure 2). The mean age of hip fracture patients is 80 years, and incidence peaks between 85-89 years of age[9,10]. Epidemiological studies predict that in 2040 aging of the general population peaks, increasing the number of hip fracture patients aged 65 and over with 8,500 to 21,218 in the Netherlands[10] (Table 1).

Table 1. Predicted increase in hip fracture incidence in patients aged ≥ 65.

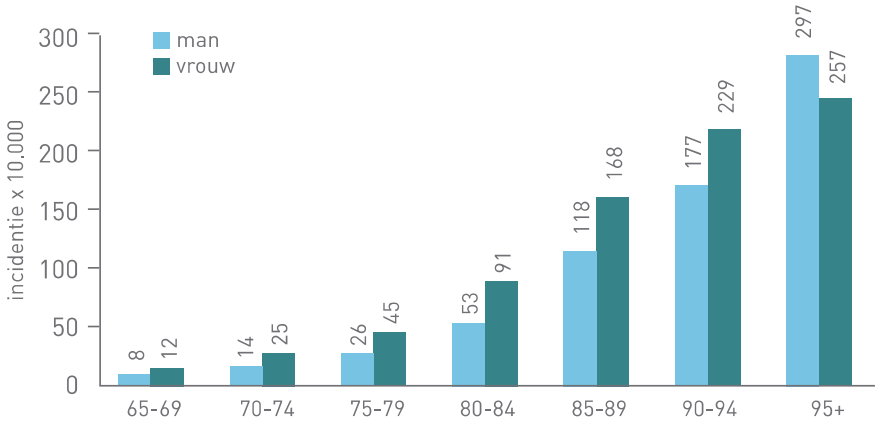
Year	Hip fracture patients aged ≥ 65	Increase relative to 2012	
2012	12,706	-	-
2015	13,502	796	6%
2020	15,241	2,535	20%
2025	17,022	4,316	34%
2030	18,885	6,179	49%
2035	20,422	7,716	61%
2040	21,218	8,512	67%

Data from landelijke traumaregistratie (LTR) April 8 2014.

A hip fracture is an extremely painful traumatic injury that nearly always requires hospital admission. The fracture pattern can be divided in intra and extracapsular fractures, both having a distinct aetiology, clinical profile, and treatment options (Figure 3). The intracapsular hip fracture or cervical neck fracture runs within the fibrous capsule, and originates from relatively low energy trauma after being predisposed by micro traumata and loss of bone mineral density i.e. osteoporosis. Depending on the clinical patient profile, and fracture displacement, these fractures can be treated by (hemi) arthroplasty or (open) reduction and osteosynthesis[7]. The extracapsular fracture or peri/subtrochanteric fracture runs outside the fibrous capsule, and although in most cases some form of loss of bone mineral density is present these fracture types result from a relatively higher energy trauma when compared to the intracapsular fracture. In general, these fracture types can only be treated by osteosynthesis[7]. Early surgical fixation or (hemi) arthroplasty with direct postoperative

mobilization remains the cornerstone of hip fracture treatment in nearly all cases, and targets preservation of pre-fracture functional status.

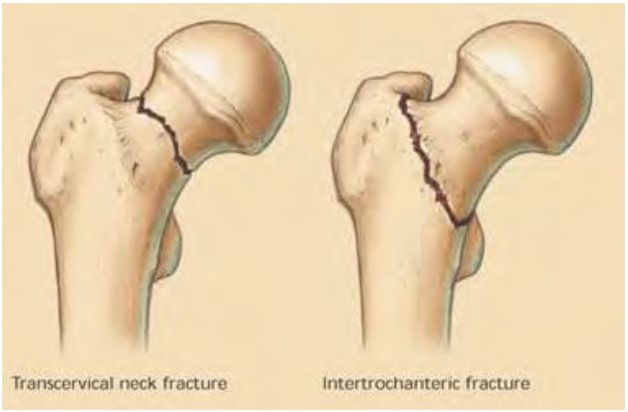
Figure 2. Incidence of hospital admissions of patients aged ≥ 65 with a hip fracture.



Data from landelijke traumaregistratie (LTR) April 8 2014.

Although early mobilization aims to preserve pre-fracture functional status, hip fractures are associated with high morbidity and complications rates, one-year mortality rates up to 29%[11,12], and significant decline in functional status[13–16]. Hip fracture pain levels are one of the highest among orthopaedic surgery[17–19], and high pain levels worsen outcome[14,15]. Special care has to be taken to avoid the most serious of complications, the onset of a delirium. With an incidence of 45%, this acute confusional state is a prevalent complication after hip fracture surgery, and although its origin is multifactorial; aged individuals yield a high risk and it strongly relates to pain[19–21]. Delirium risk is increased 9-fold in patients with high pain levels[22–24], and delirious patients take longer to ambulate, and consequently have longer length of stay[14]. The hip fracture injury causes fracture site bleeding that render 40% of admitted hip fracture patients anaemic, and this figure is increased to 93% after surgical fixation[25]. This high incidence of anaemia is problematic since it negatively impacts length of stay, readmission rates, and odds of death[25]. However, erythrocyte transfusion delays wound healing and increases risk of infection, hence the need for transfusion should be carefully considered, or avoided completely if feasible[26]. The severe pain hip fracture patients experience and the high morbidity these patients endure challenge the physician, and it has led to various attempts into providing more adequate analgesia.

Figure 3. Hip fracture types.



Pain management strategies

To date the pharmacological-based analgesics remain to cornerstone of pain treatment, but in an attempt to reduce medication-induced side effects and increase efficacy alternative administration or non-pharmacological methods are sought. First, in 30 studies comparing the use of single shot spinal anaesthesia to general anaesthesia only one randomized controlled trial (RCT) found a small analgesic benefit for spinal anaesthesia[27]. Two studies reported that patients who received general anaesthesia had 1.69 days shorter hospital admission[27]. No differences were observed in regard to the need for supplemental analgesics or overall complications (including delirium)[27]. Second, various types of peripheral (and epidural) nerve blockades were investigated. Epidural, femoral, psoas and combined nerve blocking reduce acute pain in 13 RCT's, and in 7 RCT's the need for supplementary analgesics was reduced when compared to no nerve blockade[27]. In addition, the incidence of delirium was decreased with the use of nerve blockades when compared to no blockade[27]. Third, current recovery protocols include opioid-sparing anaesthesia together with local infiltration analgesia (LIA). Opioid-sparing anaesthesia is the anaesthetic practice of using as few opioid-based analgesics as possible, by using short-acting non-opioid analgesics when possible. With LIA a large volume of local anaesthetic is infiltrated intraoperative, aiming to provide prolonged analgesia. This anaesthetic concept originated from fast-track protocols after total hip arthroplasty (THA) and total knee arthroplasty (TKA) for end-stage osteoarthritis. Although its analgesic efficacy is established after TKA[28,29], evidence of efficacy after THA for end-stage osteoarthritis is limited[28,30]. Data on LIA in hip fracture patients is sparse, and limited to non-randomized trials, where some advocate its use[31,32] and others found no effect[33]. Currently

data is too limited for a scientific rationale on whether or not to implement LIA as standard analgesic technique in hip fracture patients.

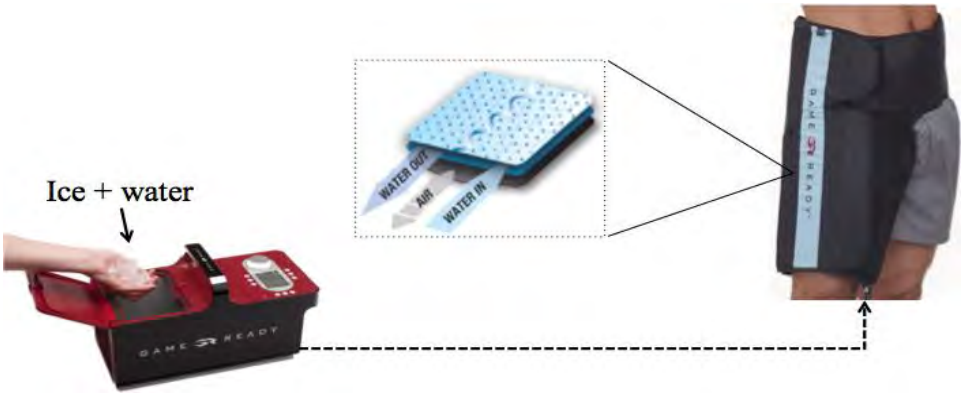
The multitude of treatment options illustrates that adequate pain control continues to be a challenge in this condition. Due to the physiological changes of ageing relative fat mass decreases, as well as muscle mass and body water, also most elderly patients experience physiological decline in organ function (such as renal or hepatic function)[34,35]. In addition, comorbidities and polypharmacy render elderly susceptible to drug interactions and adverse events[36]. These interactions and the physiological changes in body composition, and organ function predispose elderly to (opioid) analgesic medication-induced adverse effects. However the restraint that clinicians use when prescribing or dosing analgesics puts patients at risk for inferior pain treatment[36,37]. The need for adequate pain relief is evident, as inferior pain treatment is associated with delayed ambulation, which leads to an extended hospital admission, to poorer short and mid-term functional outcome and development of delirium[14,24]. These altered pharmacodynamics and kinetics narrow the therapeutic window, and taken together with the painful nature of a hip fracture this leads to increased difficulty in providing adequate analgesia to hip fracture patients[38]. Since elderly are susceptible to pharmacological-induced side effects an ideal analgesic intervention would be non-pharmacological.

Cryotherapy

Cryotherapy uses the concept of applying cold, usually in the form of ice, to injured tissue aiming to reduce pain, haemorrhage, and swelling. Cryotherapy slows nerve conduction velocity and increases pain threshold, thereby exerting a direct analgesic effect[39]. In addition, cryotherapy is suggested to reduce pain by attenuating the inflammatory cascade via a reduction in tissue metabolism[40]. By profound vasoconstriction cryotherapy reduces tissue blood flow[41], and thereby posttraumatic haemorrhage[42]. The concept of cryotherapy dates back to early days of medicine, but due to technological innovation clinicians and researchers have taken new interest in this concept as cryotherapy can now be administered by a range of commercially available machines, each having there own characteristics (Figure 4). Cold is administered through an anatomically designed wrap that covers the appropriate part of the body. The wrap allows ice-cold water to circulate over the injured tissue and withdraws body heath, some models allow air compartments to be filled in order to generate pneumatic pressure. The older models needed manual “re-chilling” of the wrap by lifting the portable control unit in order to generate a gravity-induced current. Nowadays models are fitted with a electromotor in the portable control unit that drives the ice-cold water in a continuous-flow

fashion through the wrap. If continuous-flow cryotherapy is combined with a cyclic dynamic pressure adjunct heath withdrawal is augmented. A dynamic pneumatic compressive adjunct reduces blood loss, oedema and offers a better haemodynamic profile in the deep venous and lymph system[43,44].

Figure 4. Continuous-flow cryocompression therapy applied on a right hip.



The current scientific rationale into applying continuous-flow cryocompression therapy is thin. Few studies have assessed the continuous-flow variant, as opposed to the more traditional 'static' form of cryotherapy that is more thoroughly researched. Cryotherapy is mostly applied after soft tissue trauma or after elective musculoskeletal surgery such as hip or knee arthroplasty, and anterior cruciate ligament reconstruction. Application of cryotherapy in the recovery phase of TKA results in small benefits in blood loss and short term range of motion of the knee, but does not reduce pain, analgesic demands, swelling or length of stay[42]. If cryotherapy is combined with a cyclic dynamic compression adjunct it reduces pain and postoperative knee swelling during the first three days after TKA[45]. The effect of cryotherapy after TKA is moderate, and the compression adjunct might to somewhat enhance cryotherapy' efficacy, however the same cannot be concluded from the sparse data in THA patients.

In total, only 4 studies researched continuous-flow cryotherapy in THA, and results were ambiguous. Continuous-flow cryotherapy without compression reduces analgesic demands by 50% when compared to intermittent cool pack application, while no effect is observed on blood loss[46]. Another study found non-compressive continuous-flow cryotherapy to reduce length of stay by 1.4 days, but no reduction in pain was demonstrated[47]. Continuously applied continuous-flow cryotherapy for 4 days reduces pain and analgesic medication demand, but does not attenuate postoperative blood loss[48]. Continuous-flow cryocompression therapy

attenuates postoperative haemoglobin decline, and mildly reduces morphine consumption during a 5-day admission[49]. Thus, in a limited number of low volume studies in patients that are recovering from THA for end-stage osteoarthritis diverging data exists about the effect of cryotherapy. Surprisingly, no studies have been conducted that assess the analgesic efficacy of cryotherapy in the much more painful hip fracture condition.

A major difference between hip fracture surgery and THA for end-stage osteoarthritis is that hip fracture patients have duplicate trauma. The initial fracture causes fracture-site bleeding with related inflammation, and in nearly all cases soft tissue trauma is present, which is aggravated during surgical fracture fixation[50]. As hip fracture patients have duplicate trauma, experience severe pain, and have fracture site bleeding with related inflammation, these patients are expected to benefit most from cryotherapy. However the hip joint anatomy differs significantly from for instance the knee joint, which virtually lacks an isolating subcutaneous fat layer that may have implications for cryotherapy treatment efficacy.

Currently little is known about the thermodynamic properties of cryotherapy in general, let alone in the hip joint. It is known that continuous-flow cryotherapy lowers the temperature to 23°C at 1.5 cm below the subcutaneous fat layer in healthy individuals[51]. These measurements were conducted with superficial temperature probes, so it remains unknown into what extent temperature reductions are achieved in the deeper regions surrounding the hip. It is very important to understand the thermodynamics of applied cryotherapy to the deeper tissue regions for two reasons. Currently it is not understood if a reduction in pain perception relates with temperature reductions in deep (traumatized) tissue. An established or disproven relation helps to understand the way cryotherapy exerts its analgesic effect. Two pathways can be proposed on how cryotherapy exerts its analgesic efficacy, either deeply via an interaction with tissue metabolism and immunomodulation, or superficially via an interaction on nerve conduction. Also a skin temperature benchmark should be developed that corresponds to the desired degree of analgesia or reduction in tissue metabolism that achieves clinically relevant analgesia. However regardless of the relation between pain relief and deep tissue temperature change, it is also important to explore whether deep tissue temperature is reduced by cryotherapy since low temperature is known to slow metabolism. Moreover, after surgical fixation or (hemi) arthroplasty of a hip fracture, an elevated state of metabolism is warranted to facilitate bone repair that leads to definitive healing of the fracture.

During the process of bone repair, the interruption of vascular flow activates the coagulation and formation of a fracture hematoma, which has a remarkable angiogenic capacity[52]. The interruption of vascular flow causes an hypoxic state, and hypoxia is a key

factor in bone repair[52]. Vascular endothelial growth factor (VEGF) is a bone-metabolism cytokine that is upregulated by hypoxia, and stimulates the proliferation and chemotactic migration of osteoblast precursor cells[53]. Being a powerful inducer of angiogenesis, VEGF is important for the later stages of bone healing when vascularization of the callus is warranted[54]. VEGF production decreases by 30% in hypoxic retinal pigment epithelial cells exposed to moderate hypothermia[55]. Hypothermia reduces osteoblast proliferation and differentiation while promoting osteoclast function in cultured rat calvariae[56]. Taken together, these findings of possible adverse effects of hypothermia on VEGF production, osteoblast and osteoclast function warrants further investigation of induced hypothermia effects on early stages of bone healing.

Aim of the present thesis

Based on the considerations above, this thesis addresses four major questions. *First.* What is the current evidence in the application of continuous-flow cryotherapy with or without a compression adjunct in various types of surgical-induced musculoskeletal trauma? *Second.* What is the efficacy of continuous-flow cryocompression therapy in the acute postoperative recovery phase of hip fracture surgery in regard to analgesia, postoperative blood loss, short-term functional outcome, and is it feasible to use in daily practice? *Third.* In what degree does continuous-flow cryocompression therapy reduce deep tissue temperature, and are pain and deep tissue temperature related in the hip? *Fourth.* How does hypothermia affect various bone healing parameters in a stem cell model, by using hypoxia as surrogate for a fracture?

The specific aims of this thesis are:

- To provide a detailed overview, and to summarize and meta-analyse the available evidence of various application modalities of continuous-flow cryotherapy applied after surgery for musculoskeletal trauma (**Chapter 2**);
- To explore the feasibility and efficacy of continuous-flow cryocompression therapy in the postoperative recovery phase of THA for end-stage osteoarthritis in a pilot-study (**Chapter 3**);
- To establish the analgesic efficacy, and the effects of continuous-flow cryocompression therapy in the postoperative recovery phase of hip fracture surgery on postoperative blood loss, short-term functional outcome parameters, and feasibility (**Chapter 4 and 5**);

- To define deep tissue temperature change by on continuous-flow cryocompression therapy after hip fracture surgery and to assess an association between deep tissue temperature change and pain (**Chapter 6**);
- To determine whether hypothermia modulates proliferation, apoptosis, nitric oxide production, VEGF gene and protein expression, and osteogenic/chondrogenic differentiation of human mesenchymal stem cells under hypoxia (**Chapter 7**).

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Chapter 2

The efficacy of continuous-flow cryotherapy in the acute recovery phase of lower extremity surgery

OA Orthopaedics 2014 May 02;2(1):9

Nick C. Leegwater, Inger N. Sierevelt, Peter A. Nolte

Abstract

Context: Continuous-flow cryotherapy is a new cold application modality that is applied in the postsurgical recovery phase of musculoskeletal lesions of the lower extremity. Although its use is widespread and is believed to exert analgesic effects and to enhance recovery, scientific evidence into justifying its application is lacking.

Objectives: We conducted a systematic review with meta-analyses of randomized, controlled clinical trials assessing the short-term analgesic efficacy of continuous-flow cryotherapy, with and without intermittent or static compression, in the acute recovery phase after surgery for musculoskeletal lesions of the lower extremity. We also assessed narcotic analgesic medication; drain output; decline in haemoglobin; erythrocyte transfusion incidence; hospital admittance time; complications and a cost-benefit analysis.

Evidence acquisition: Six electronic databases were systematically searched in February 2014. Inclusion criteria were: fluid-based continuous-flow cryotherapy, randomized or quasi-randomized design and application directly after surgical repair of musculoskeletal lesions of the lower extremity. Effect sizes, relative risk, and confidence intervals were calculated to compare continuous-flow cryotherapy to: 1) no cryotherapy, 2) static compression, 3) intermittent- or 4) sham cryotherapy.

Evidence synthesis: 16 studies met the inclusion criteria. Ten studies were included in meta-analyses after GRADE-assessment. A total of 1,077 participants: 264 total hip arthroplasties, 773 knees (312 total knee arthroplasties, 408 anterior cruciate ligament reconstructions, and 53 arthroscopies) and 40 foot fractures/ligament injuries were included. No statistical significant pooled effect in favour of continuous-flow cryotherapy was observed. Trends were observed that supported a decline in narcotic use and lesser drain output in favour of continuous-flow cryotherapy.

Conclusions: The efficacy of continuous-flow cryotherapy on outcomes in the postsurgical recovery phase of musculoskeletal lesions of the lower extremity is unclear. Future research is needed to confirm the observed mild advantage in narcotic use.

Introduction

Soft tissue trauma, either traumatically or surgically acquired, induces an inflammatory response that involves increased leakage of vascular contents, resulting in haematoma and oedema. Tissue trauma can lead to secondary cell injury through associated hypoxia due to reduced functional capillary density[1,2].

For a long time cryotherapy has been an adjunct in the recovery of acute musculoskeletal trauma and after orthopaedic and/or surgical interventions, its use dates back to as early as 2500 BCE[3]. Through a reduction in tissue metabolism, cold-induced vasoconstriction and concomitant stimulation of skin-thermoreceptors, cryotherapy attenuates the inflammatory cascade, provides short-term analgesic effects and reduces blood loss and oedema[1,2,4-12].

Though its theoretical basis has remained the same over the years its application has changed, especially since the introduction continuous-flow cryotherapy (CFC) machines. This fundamental advance in the application of cryotherapy results in faster and better cooling of tissues[13,14]. Albrecht et al. compared the use of a continuous-flow apparatus to the use of intermittent application of cryotherapy and found a skin temperature reduction of 12°C compared to 1°C respectively[14]. The cryotherapy induced analgesia commences between 12°C and 15°C[13,14].

The intermittent cyclic compression function, which is embedded in the latest generation of cryotherapy machines, facilitates external pressure by which it can potentially further decrease posttraumatic wound oedema. It also maintains a flow gradient in the deeper venous system and avoids stasis, a known risk factor for thrombosis.

Within sports the analgesic capabilities of cryotherapy are well known, in all professional levels cryotherapy has been applied after musculoskeletal lesions for many years. Though its use is widespread and is considered to be a valuable adjunct by sportsmen and their trainers, clear evidence is not as straightforward[11,15-17].

Numerous clinical studies and several review authors[11,15-17] have sought to compare the different application forms of cryotherapy: (crushed) ice bags, intermittent and continuous-flow cryotherapy machines. However the efficacy of the next-gen, solely continuous-flow, cryotherapy machines have not yet been clearly established within a systematic review with meta-analysis.

The purpose of this systematic review is to compare randomized, controlled clinical trials that assess the efficacy of continuous-flow cryotherapy machines, with and without the adjunct of intermittent or static compression after surgery for musculoskeletal lesions of the lower extremity.

Methods

This review was conducted in accordance with the Cochrane Collaboration guidelines for systematic reviews and meta-analyses; the PRISMA statement and GRADE quality assessment were used during drafting of the manuscript.

Eligibility criteria

Study types

The search strategy for this meta-analysis was limited to randomized controlled trails (RCT) and quasi-randomized clinical trials (q-RCT). The intervention of interest was fluid-based continuous-flow cryotherapy (with or without (cyclic) compression) that was applied in the acute postoperative phase of patients recovering from surgical repair for musculoskeletal lesions of the lower extremity. The acute phase was defined as the first 72 hours (h) postoperative.

Types of patients

Inclusion was limited to studies on patients over 18 years of age with surgery for musculoskeletal lesions of the lower extremity. Indication for surgery could consist of: tendon, ligamentous or bone injury and end-stage osteoarthritis necessitating arthroplasty.

Types of intervention

Inclusion focused on articles on fluid-based continuous-flow cryotherapy (with or without (cyclic) compression). No restrictions were imposed towards control group(s) aspect which could consists of: (I) no cryotherapy, (II) static compression, (III) intermittent cryotherapy or (IV) sham cryotherapy.

Types of outcome

The primary outcome measure was (I) pain assessed in the first 72 h postoperative by use of a Visual Analogue Scale (VAS) or Numerical Rating Scale (NRS). Other outcomes were (II) postoperative blood loss (drain output in ml the first 24 h postoperative), (III) postoperative haemoglobin level (mmol/l), (IV) erythrocyte transfusion incidence (incidence and number of transfused packed cells), (V) narcotic analgesic medication usage during admission (where possible opioid forms were recalculated to a single form[18] or equianalgesic dose (EAD)[19,20], (VI) hospital admittance time (days), (VII) (cryotherapy related) complications and (VIII) cost-benefit analysis.

Search and data extraction strategy

Reviewers

Studies were independently selected by 2 reviewers (NL and IS) via a systematic search of electronic databases; Embase, Medline through Pubmed.gov, Cochrane, Web of Science, PEDro and CINAHL. All were last accessed at the 21st of February 2014. The MEDLINE search is available from pubmed.gov[21]. The 'related articles' function in MEDLINE and EMBASE and the reference lists of included articles were utilized to maximize search sensitivity. Only published articles were reviewed. Abstracts from meetings, letters to the editor, unpublished reports and review articles were excluded. All languages were considered, if translation was possible.

Study selection and data extraction

Two reviewers (NL and IS) independently screened all retrieved studies based on title and abstract. Potentially eligible references were retrieved as full-text. A single reviewer (NL) extracted the study characteristics and data by using data extraction sheets based on the Cochrane Consumers and Communication Review Group's data extraction template. A second author (IS) verified the extracted data and previously derived analyses. In case of disagreement, a third party (PN) was consulted. If necessary, authors were contacted to retrieve additional data from included articles; one[12] responded and provided us with additional numerical data.

Studies with multiple intervention groups were pooled into one group, if this was deemed appropriate. Studies comparing several groups at different temperature settings were deemed appropriate to pool. Pooling was performed according to the Cochrane handbook methods[22]. Reviewers were not blinded for author or publishing journal.

Risk of bias assessment

Two authors (NL and IS) independently assessed methodological quality of the included studies. This critical step in the review process evaluates the risk of bias; the risk of bias assessment was performed with use of the Cochrane risk of bias assessment tool.

Quantitative analysis

A data-driven approach was employed for the synthesis by using RevMan (v5.1.2). Due to considerable expected clinical heterogeneity, sub-analyses were performed for studies on different interventions. Analysis was performed using random effects model. Standardized

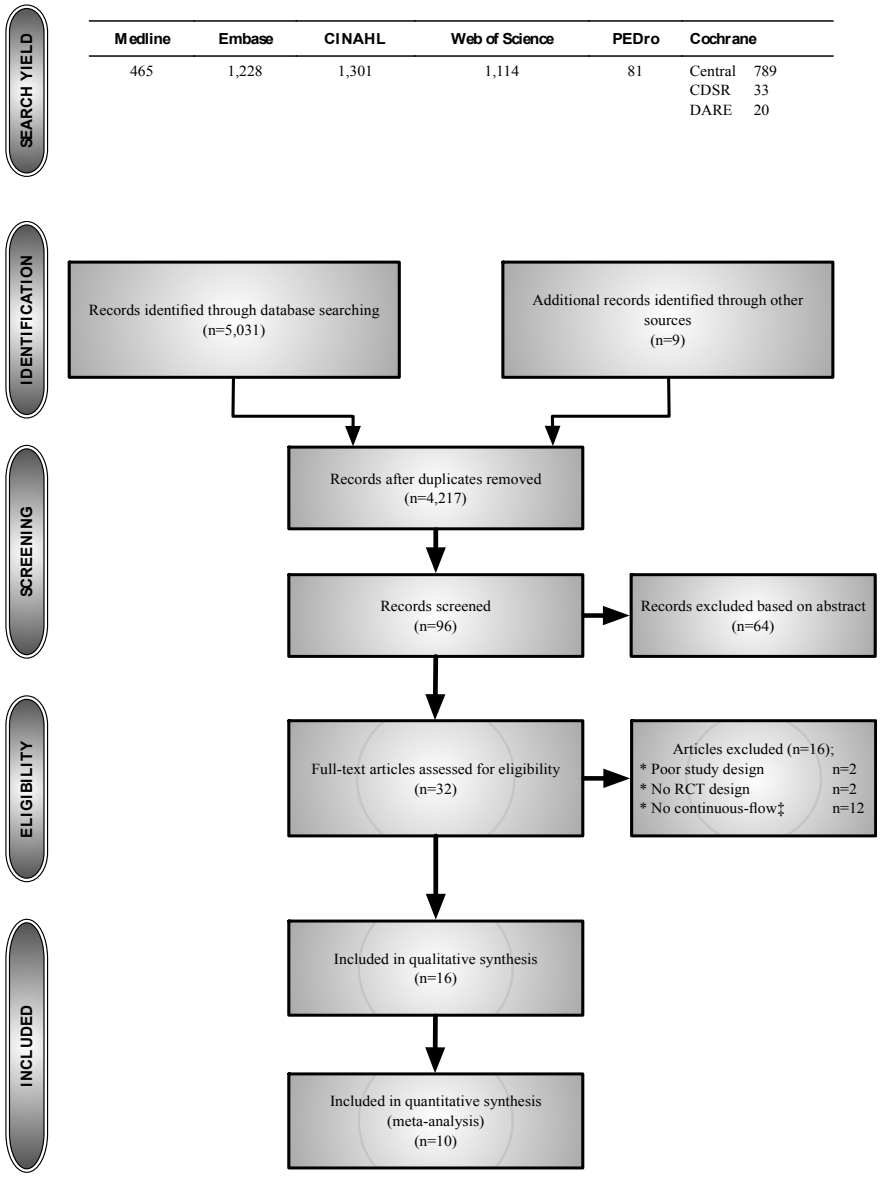
mean differences with 95% confidence intervals were calculated where differences in outcome measurement were present. Significant heterogeneity existed in the reporting of analgesic use. Nearly all studies reported tablets or units parenteral narcotic analgesics in different forms during stay as analgesic outcome. Other analgesics e.g. diclofenac were rarely stated and were omitted from analyses. Three studies[23-25] reported total narcotic analgesic usage in an EAD. This proportion allows narcotic analgesics, administered in various ways, to be recalculated to a single form: an EAD[19,20]. Recalculation of several included studies[12,23,25-28] was achieved with the following proportion: 75 mg intramuscular Demerol equals 10 EAD; 30 mg codeine equals 1.5 EAD; 1 mg of parenteral morphine equals 1 EAD and 1 tablet of hydrocodone 5 mg equals 1.5 EAD. Mean differences with 95% confidence intervals of the equianalgesic doses were calculated in the meta-analysis 'narcotic use'.

Heterogeneity was explored by visual checking of overlapping confidence intervals (eyeball-test). Furthermore, formal testing was achieved with the Cochran chi-square test (Cochran Q). The Higgins method of consistency measurement was applied and expressed as I^2 value, studies were judged to be heterogeneous if $I^2 \geq 80\%$[29].

In case studies compared multiple similar but non-identical interventions (such as multiple temperature settings) groups were merged according to the Cochrane handbook described methods[22]. Where only means were reported with complementary ranges, the method described by Walter et al. was used to estimate the standard deviation (SD)[30].

In case a mean and confidence interval were reported the SD was calculated by dividing the length of the confidence interval by 3.92 and multiply this figure by the square root of the sample size (n)[22]. Furthermore when pooling the SD in a single group for different outcomes e.g. oral and parenteral morphine, the square root of the sum of the two squared SD's was taken to calculate the new SD. The GRADE quality rating system was used to judge articles' quality of evidence in order to draw adequate conclusions[31].

Figure 1. Search yield and study selection flowchart.



‡ Studies did not use continuous-flow cryotherapy, or did not state which cryotherapy was used.

Results

Included studies

Searching of Embase, Medline, Cochrane, Web of Science, PEDro and CINAHL provided a total of 5,031 studies. Three additional references were selected using the reference lists of eligible articles. 4,217 articles remained after duplicates were removed. Based on their title and abstract, 96 articles abstracts were read, of which 32 were retrieved in full-text. It appeared that 16 articles did not meet the inclusion criteria. The remainder 16 articles were included in the review, 10 were deemed appropriate and feasible to include in meta-analysis (Figure 1).

A total of 16 studies were included, amongst were 2 German articles. Study characteristics are displayed in Table 1. Besides the United States, the studies were conducted in Australia, Sweden, The Netherlands, Japan and Germany between 1989 and 2012. A total of 1,077 participants: 264 total hip arthroplasties (THA), 773 knees (312 total knee arthroplasties (TKA), 408 anterior cruciate ligament (ACL)-reconstructions, 53 arthroscopies) and 40 footfractures/-ligament injuries were included. Sample sizes ranged from 21 to 208.

The results of risk of bias assessment are displayed in Table 2. The section 'patient blinding' is omitted from the table because it is the authors' opinion that no true blinding can exist when a cryotherapy machine is used. Scarcella et al.[32] achieved blinding in some degree since their patients were unaware that a study was in progress, however performance bias is still likely.

Incidence of outcome measures

Nine out of 16 studies (56%) reported on our primary outcome parameter pain[12,14,23,26,28,33-36]. Most used the VAS to report pain, Woolf et al used a NRS, range from 0 – 5. Significant difference existed towards the mode of measuring the VAS-pain. In several studies it was unclear to what VAS-pain was measured, e.g. overall, worst or a single measurement.

In five studies[12,23,25,27,28] the calculation to an EAD was feasible. Saito et al. reported on intrathecal mepivacaine, no EAD could be calculated for this narcotic analgesic. Table 3 displays both the EAD-based meta-analysis result and the study-reported narcotic analgesics.

Half of the six studies [25,28,37] that reported drain output did not state the time of drain removal. Nor did they state whether the drain output was cumulative or not. Leegwater et al. and Albrecht et al. reported postoperative haemoglobin decline. The data of Albrecht et al. were reported in mg/dl, which were converted to mmol/l to facilitate comparison.

Treatment characteristics

Various temperature settings were employed, ranging from 4 to 21°C. Daniel et al. and Ohkoshi et al. used different temperatures settings across multiple intervention groups. Four studies used cyclic compression as an adjunct; Leegwater et al. used various cyclic pressure settings up to 75 mmHg, the other three studies[28,33,37] up to 30 mmHg. The application time was fairly consistent, most studies applied cryotherapy continuously during admission. Leegwater et al. and Woolf et al. used an intermittent regime. In all but four studies[14,34,37,38] the cryotherapy was continued more than 72 h, mostly until patient's discharge.

Treatment effectiveness

Postoperative pain

The following groups were combined; the 5 and 10 °C groups in Ohkoshi et al.; the 4°C, 7°C, 13°C and 21°C plus control groups of Daniel et al. There was very low quality evidence that CFC slightly decreases acute pain in the first 72 h (Table 2). Two[14,26] out of the six studies that were not included in the meta-analysis and compared CFC to no cryotherapy showed a statistical significant advantage in pain relief of nearly 50% in the first 48 h. In the 5°C group Ohkoshi et al. found a small significant increase in pain perception. The remaining studies demonstrated a small clinical advantage that was statistically non-significant.

Narcotic analgesic use

Fourteen out of 16 studies reported narcotic analgesic use. Continuous-flow cryotherapy significantly reduced analgesic demands in 5 studies[26,27,33,34,37], Saito et al. demonstrated a reduction of almost 50%; 295 mg vs. 489 mg of intrathecal mepivacaine use in 7 days. Ohkoshi et al. employed a 5°C and 10°C temperature setting, they found contrasting results with significantly lower narcotic analgesic use in the 10°C comparing to no difference in the 5°C. Studies were pooled using recalculated EAD as described above. Overall, low quality evidence exists for an advantage in narcotic use in favour of CFC (Table 2).

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Blood loss

Low quality of evidence supports the finding that CFC reduced drain output when data of 6 studies was pooled (Table 2). When considered separately, only Ohkoshi et al. demonstrated a statistically significant decline in their 5°C group of almost 50%, 51.7 ml vs. 97.9 ml at postoperative day 1 (POD1). In the 10°C group however blood loss was increased (78.3 ml vs. 32.6 ml, $p>0.05$). Moderate heterogeneity existed due to the study of Radkowski et al. (I^2 74%, $p=0.002$). Explanations can be the lower temperature setting than in the other two TKA studies[25,37]. Furthermore Radkowski et al. implanted a TKA while the other 2 implanted an unicondylar prosthesis, the use of regional nerve blocks can also have a degree of effect due to vasodilatory effects. The timing of outcome measurement was unknown in the other two studies.

Table 1. Characteristics of studies.

Author (year)	Surgery type	I/C, *n*	Age, yrs[b]	Device	Temperature setting	Compression	Application	Control group and co-intervention	Outcomes of interest
Albrecht '97	THA	72/70	69	Criojette	4°C	No	Continuous for 48 h	Intermittent cool packs	* VAS pain * Drain output * Hb decline
"	TKA	35/31	"	"	"	"	"	", CPM + EA	"
Scarcella '95	THA	28/22	I: 70, C: 70	Hot/Ice Thermal Blanket	5°C	No	Continuous until discharge	No cold	* Analgesic use * Length of stay
"	TKA	12/12	"	"	"	"	"	"	"
Leegwater '12	THA	12/15	I: 66, C: 68	Game Ready System	4°C	Cyclic, 5-75 mm Hg	4x/day for 30 min during 5 days	Static compression	* VAS pain * Analgesic use * Hb decline
Saito '04	THA	22/23	I: 59, C: 59	Icing System 2000	5°C	No	Continuous for 4 days	No cold, EA for 24 h	* VAS pain * Analgesic use * Drain output
Radkowski '07	TKA	28/36	I: 64, C: 67	ThermoTek Solid State recirculating	7°C	No	Continuous until discharge	Cryotherapy at 24°C, PCA-iv	* VAS pain * Analgesic use * Drain output
Ivey '94	TKA	28/30	I: 64, C: 67	Hot/Ice Thermal Blanket	10, 15°C	No	Continuous for 72 h	Cryotherapy at 21°C	* Analgesic use
Walker '91	UKA	15/15	I: 75, C: 70	"Cooling pad"	10-13°C	No	Continuous for 72 h	No cold, CPM	* Analgesic use * Drain output * Length of stay
Hölmstrom '05	UKA	23/17	I: 68, C: 72	Cryo/Cuff with AutoChill	10-15°C	Cyclic, 30 mm Hg	Continuous for 48 h	No cold	* VAS pain * Analgesic use * Drain ouput
Barber '98	ACL-R	51/59	34	Cryo/Cuff with AutoChill	2-10°C	Cyclic, 30 mm Hg	Continuous for 72 h	No cold, CPM, IA morphine	* VAS pain * Analgesic use
Cohn '89	ACL-R	51/59	24	Hot/Ice Thermal Blanket	10°C	No	Continuous until discharge	No cold	* Analgesic use * Length of stay

Daniel '94	ACL-R	16/30/13/30[*]	27	DuoTemp	4, 7, 13, 21°C	No	Continuous for 72 h	No cold, CPM	* VAS pain * Analgesic use * Length of stay
Dervin '98	ACL-R	(40/38)	I: 31, C: 27	Cryo/Cuff with AutoChill	Unknown	Cyclic, 30 mm Hg	Continuous until discharge	Room water temp cryotherapy, CPM	* VAS pain * Analgesic use * Length of stay * Drain output
Konrath '96	ACL-R	27/27	I: 27, C: 27	Polar Care 500	10-15°C	No	Continuous until discharge	No cold	* Analgesic use * Drain output * Length of stay
Ohkoshi '99	ACL-R	7/7/7[*]	22	Icing System 2000	5, 10°C	No	Continuous for 48 h	No cold	* VAS pain * Analgesic use * Drain output
Woolf '08	Arthroscopy	24/29	42	Polar Care 500	Unknown	No	At night during 4 days	Ice pack + static compressive dressing, IA bupivacaine	* VAS pain * Analgesic use
Stöckle '95	Footfractures[a]	20/20	I: 32, C: 33	Polar Care 500	Unknown	No	Continuous preop until POD 4	Intermittent cool packs	* VAS pain * Analgesic use

[a]: Also ligamentous injury; [b]: Presented as mean. I: intervention; C: control; THA: total hip arthroplasty; TKA: total knee arthroplasty; UKA: unicondylar knee arthroplasty; ACL-R: anterior cruciate ligament reconstruction; CPM: continuous passive motion; EA: epidural analgesia; PCA: patient controlled analgesia; IA: intra-articular; VAS: visual analogue scale.

Table 2. Risk of bias assessment.

	Random sequence generation	Allocation concealment	Blinding of treatment providers	Blinding of outcome assessors	Incomplete outcome data	Selective reporting	Group similarity at baseline	Other source of bias
Albrecht '97	?	?	+	?	-	?	-	?
Barber '98	+	+	+	+	-	-	?	?
Cohn '89	?	?	+	?	-	-	?	?
Daniel '94	+	+	+	?	?	-	-	?
Dervin '98	+	+	+	?	-	-	-	?
Hölmstrom '05	?	?	+	?	-	-	-	?
Ivey '94	+	?	+	?	?	?	-	?
Konrath '96	-	+	?	?	-	?	-	?
Leegwater '12	?	-	+	?	-	?	-	?
Ohkoshi '99	?	?	+	?	-	-	?	?
Radkowski '07	-	-	+	-	-	-	-	-
Saito '04	?	?	+	?	-	+	-	?
Scarcella '95	?	?	+	?	?	?	?	?
Stöckle '95	+	+	+	?	-	-	?	?
Walker '91	?	?	+	?	-	-	?	?
Woolf '08	+	+	+	?	-	-	-	?

Length of stay

Continuous-flow cryotherapy did not seem to reduce hospital admittance time in most of the studies. Seven of the 8 studies reporting length of stay found similar figures, only the findings of Scarcella et al. were significantly in favour of THA patients, with admittance time 1.4 days shorter than the 10.3 days in the control group. No signs of mean difference in admission time were observed when data of four studies [23,25,28,36] were analyzed (Table 3). Evidence was very low. Because analyzed trials had a major spread in regard to publication year, evidence was downgraded. Other factors are likely to influence length of stay (e.g. fast-track).

Adverse events

All but Ohkoshi et al. reported on adverse events, in general or cryotherapy related. Of all 596 intervention participants only 9 patients required discontinuation because of discomfort or cold urticaria. Cohn et al. reported one case of transient peroneal nerve palsy due to an accidentally long application of an ice bag for 40 minutes, the palsy resolved spontaneous after cessation of therapy.

Cost-benefit analysis

None of the included studies made a cost-benefit analysis, only Konrath et al. and Stöckle et al. reported the costs of a cryotherapy machine which are $ 225, - or around € 230, -.

Only Leegwater et al. and Albrecht et al. reported postoperative haemoglobin levels. Leegwater et al. demonstrated a 1.79 mmol/l decline vs. 2.34 mmol/l in the control group ($p<0.05$) at POD 1. At POD 3 compared to the preoperative value, the advantage persisted in a lesser degree, but lost significance. Albrecht et al. measured haemoglobin at POD 2 and found a mild advantage of 0.3 mmol/l that was similar in TKA and THA but not significant.

Table 3. Summary of findings.

Intervention	Surgery	1st Author	Temp. setting	Acute pain[b] (I vs. C)	Analgesic use	Drain output (ml)	Length of stay (days)	Hb decline (mmol/l)
Continuous-flow cryotherapy vs. no cryotherapy	THA	Saito	5°C	At POD1: 2 vs. 4* At POD2: 1 vs. 3*	295 vs. 489 mg* *Mepivacaïne use in 7 days*	**At POD1: 733 vs. 755‡**	-	-
	THA	Leegwater	4°C	**At POD1: 3.5 vs. 4‡**	**84.7 vs. 100 mg‡** ***Oramorph during admission***	-	**4.75 vs. 5.0‡**	At POD1: 1.79 vs. 2.34*,*f* At POD3: 2.16 vs. 2.63‡,*f*
	ACL-R	Barber	2 – 10°C	At POD2: 5.61 vs. 5.88‡	1.49 vs. 2.85** *Vicodin use at POD2*	-	-	-
	ACL-R	Konrath	10 – 15°C	-	46.3 vs. 38.6‡ *Total EAD*	At POD1: 114 vs. 116‡	1.2 vs. 1.1‡	-
	ACL-R	Ohkoshi	5°C	**76.7 vs. 65.7*,*c***	1.25 vs. 1.5‡ *No. diclofenac 25 mg in 48 h*	**At POD2: 51.7 vs. 97.9****	-	-
	ACL-R	"	10°C	**34.7 vs. 65.7*,c**	0.7 vs. 1.5* *No. diclofenac 25 mg in 48 h*	**At POD2: 78.3 vs. 32.6‡**	-	-
	ACL-R	Daniel	4°C	4.1*d*,‡	**5.5‡**	-	3.1‡	-
			7°C	4.6*d*,‡	**5.3‡**		3.1‡	
			13°C	5.7*d*,‡	**4.8‡**		3.3‡	
			21°C	4.9*d*,‡	**4.4‡**		3.2‡	
			No cold	4.1*d*,‡	**10.2‡** ***Oral narcotics***		3.2‡	
	UKA	Walker	10 – 13°C	-	**118 vs. 164‡** **Total EAD**	**642 vs. 688‡** **Timing unknown**	**7.9 vs. 8.1‡**	-
	UKA	Hölmstrom	10 – 15°C	-	**277 vs. 361‡** ***Morphine, timing unkown***	**277 vs. 361‡** **Timing unknown**	-	-
	ACL-R	Cohn	10°C	-	**5.2 vs. 11.2*** **Total Demerol, mg/kg**	-	**3.5 vs. 3.5‡**	-

Continuous-flow vs. intermittent cryotherapy	THA	Albrecht	4°C	At POD1: 2 vs. 4** At POD2: 2.05 vs. 3.83**	-	At POD2: 1676 vs. 1542‡	-	At POD2: 1.07 vs. 1.33‡,*f*
	TKA	Albrecht	4°C	At POD1: 2.65 vs. 7** At POD2: 2.34 vs. 6.5**	-	At POD2: 947 vs. 1177‡	-	At POD2: 0.91 vs. 1.27‡,*f*
	Foot-fractures[a]	Stöckle	NR	-	Data NR Stated no difference	-	-	-
	Arthroscopy	Woolf	NR	**At POD2: 2.64 vs. 2.95‡,*e***	-	-	-	-
	THA	Scarcella	5°C	-	4.75 vs. 4.75‡ *Total Meperidine mg/kg*	-	8.9 vs. 10.3*	-
Continuous-flow cryotherapy vs. sham	TKA	"	"	-	4.14 vs. 4.44‡ *Total Meperidine mg/kg*	-	8.6 vs. 10.1‡	-
	TKA	Radkowski	7°C	At POD1: 6.0 vs. 5.5‡ At POD2: 7.1 vs. 6.3‡	At POD1: 7.1% vs. 5.6%‡ At POD2: 46% vs. 25%‡ *Patients using narcotics*	**At POD2: 448 vs. 519‡**	-	-
	TKA	Ivey	10°C	-	1.6 vs. 1.3‡ *Total morphine, mg/h*	-	-	-
	TKA	"	15.5°C	-	1.4 vs. 1.3‡ *Total morphine, mg/h*	-	-	-
	ACL-R	Dervin	NR	**30 vs. 25‡**	**0.37 vs. 0.35‡** ***Total morphine, mg/kg*** **3.86 vs. 3.44‡** ***No. of codein 30 mg tablets***	**335 vs. 348‡** **Timing unknown**	**60 vs. 55 h‡**	-
Meta-analyses	-	-	-	SMD -0.01 [-0.23, 0.22] *p*=0.96	MD -7.04 [-19.40, 5.32] *p*=0.26	SMD -0.46 [-0.96, 0.04] *p*=0.07	MD 0.07 [-0.16, 0.31] *p* 0.53	-
Heterogeneity	-	-	-	0%, *p*=0.46	62%, *p*=0.03	74%, *p*=0.002	0%, *p*=0.64	-
Studies, *n* (I/C)	-	-	-	5 (149/161)	5 (152/167)	6 (142/136)	4 (93/95)	-
GRADE	-	-	-	Very low *1, 2, 3* Woolf compared to intermittent cryotherapy	Low *1, 2*	Very low *1, 2, 4* Timing of measurement of drain output unknown	Very low *1, 2, 3* Cohn compared to intermittent cryotherapy; Spread of publication year	-

Bold outcomes are entered in meta-analysis. ‡: not significant; *p<0.05; **p<0.01; NR: not reported. □: decline from preoperative value. EAD: equianalgesic dose. ®: Most severe pain in 48hrs. †: overall VAS during admission. : for various indications, amongst are meniscal repair, removal of corpora aliena, lateral retinacular release (no ACL or PCL reconstructions). 1: downgraded due to indirectness. 2: downgraded due to <400 participants and/or broad confidence intervals. 3: downgraded due to high risk of bias in included studies. 4: downgraded due to inconsistency.

Table 3. Summary of findings *(continued)*.

Intervention	Surgery	Author	Conclusions
Continuous-flow cryotherapy vs. no cryotherapy	THA	Saito	1) Pain less the first four days 2) Less Mepivacaïne use 3) Intervention group pain free at POD3, controls pain free at POD5
	THA	Leegwater	1) Less morphine use 2) Less haemoglobin decline
	ACL-R	Barber	Pain scores always higher in controls
	ACL-R	Konrath	No differences
	ACL-R	Ohkoshi	1) 5°C cryotherapy reduces blood loss and increases pain perception in a small degree 2) 10°C cryotherapy alleviates pain and has no effect on blood loss
	ACL-R	Daniel	No difference in hospital stay, pain medication use or pain scores
	UKA	Walker	1) No difference in length of stay or drain output 2) Total narcotic consumption did not differ 3) Less oral analgesic consumption in intervention group
	TKA	Albrecht	1) Analgesic demand was 2.5x higher in controls 2) Three intervention patients required no narcotics the first 48h
	UKA	Hölmstrom	1) No difference in VAS, drain output or swelling 2) Significantly less morphine consumption in intervention group
	ACL-R	Cohn	1) At POD 2, 50% of intervention group only used oral narcotics compared to 30% in control group 2) Intervention group used 53% and 67% less demerol and vistaril respectively 3) Mobilisation was better in the intervention group
Continuous-flow vs. intermittent cryotherapy	THA	Albrecht	No difference in drain output
	TKA	Albrecht	1) Analgesic demand was 2.5x higher in controls 2) Three intervention patients required no narcotics the first 48h
	Foot-fractures[a]	Stöckle	1) No difference in analgesic consumption 2) Cryotherapy reduces swelling
	Arthroscopy	Woolf	1) No difference in pain intensity 2) @ POD 2 35.7% of intervention patients reported mild nocturnal pain compared to 5.9% 3) No difference in functional activity impairment
Continuous-flow cryotherapy vs. sham	THA	Scarcella	Intervention group subjects discharge 1.4 days faster
	TKA	"	No differences in narcotic use or length of stay
	TKA	Radkowski	1) No difference in pain 2) Less nocturnal pain 3) No difference in drain output
	TKA	Ivey	No difference in morphine consumption
	ACL-R	Dervin	No significant differences in regard to drain output, pain, narcotic use or length of stay

Discussion

To our knowledge we are the first authors that evaluate the effect of solely continuous-flow based cryotherapy machines. As these machines are considered the new standard in cryotherapeutical applications, it is important to understand their relevancy when considered for treatment in the acute recovery phase of musculoskeletal trauma. During reviewing the PRISMA, Cochrane risk of bias assessment and GRADE quality assessment were carefully followed, providing clinicians, patients and scientists with transparent results for health-care decision-making. Overall, evidence is too limited to take an adequate stance on the efficacy of continuous-flow cryotherapy application in the postoperative phase.

Most of the 16 included trials agree on a small analgesic effect, while meta-analysis provided very low evidence of no apparent effect when CFC is applied. Most studies did not report what kind of VAS-pain was measured; VAS-pain on movement or in rest, a single VAS-pain measurement, average daily VAS-pain or worst VAS-pain. Ohkoshi et al. and Radkowski et al. reported the latter, while Daniel et al. measured the overall VAS-pain during stay. Furthermore confounding is present as Woolf et al. and Barber et al. injected analgesics intra-articular after surgery as local anaesthetic. Intravenous PCA-pumps were used in four studies[12,28,36,39]. When a patient controlled mode of narcotic analgesic administration is used, one can hypothesize that the threshold to administer the analgesics is lower and thus might be higher. Continuous passive motion machines were used in six studies[14,23,25,28,33,37]. The mode of anaesthesia was stated in a few studies; loco regional blocks take some time to wear off and could have a profound effect on pain perception the first few hours.

It is highly likely - if not certain - that the perceived pain levels are confounded by a second outcome parameter: (narcotic) analgesic medication. Hospital protocols generally focus on keeping VAS-pain levels to a minimum with supplementary (narcotic) medication. Most studies did not report on other than narcotic analgesic medication. It is generally accepted to first use acetaminophen and NSAID's before resorting to narcotic analgesics. The assumption was made that this was the case in the included studies but that other medication than narcotics were simply not reported in the majority. Therefore we restricted analysis to narcotics. We found low quality of evidence for a reduction in narcotic analgesic use if cryotherapy machines are used. However the clinical relevance of these results is hard to determine. The 50% reduction that some studies reported are not unanimously found in other studies and seems an overestimate of the effect size. However a reduction of narcotic consumption certainly does contribute to a lesser degree of drug induced side effects,

especially in frail patient populations such as elderly. No trial to date has ever evaluated the efficacy of CFC in such a population.

Apart from the 5°C group in Ohkoshi et al. all trials confirmed either a reduction in drain output, or no effect at all. Very low evidence suggests a moderate advantage of CFC, which was near significant. Interpreting these figures should be done with caution, as most studies did not report the mode of anaesthesia. Loco regional spinal anaesthesia is known for its vasodilatory effects in a longer time span compared to general anaesthesia. The time of drain removal was poorly reported, it was not stated in 3 out of 8 studies. The remainder did not state the hour of removal i.e. in the morning or evening. Therefore significant spread i.e. indirectness exists for which the evidence was downgraded.

Postoperative haemoglobin decline was only measured in two studies, Leegwater et al. demonstrated a small statistical significant reduction the 1st POD, but lost significance later on. Albrecht et al. demonstrated similar but non-significant differences. None reported on erythrocyte transfusion necessity, an important patient related outcome. Colleagues Adie et al. did include two RCT's reporting on transfusion incidence but findings were comparable between groups in TKA[15].

Very low evidence suggested no difference was present when length of stay was analyzed; only Scarcella et al. demonstrated a statistical significant advantage of more than a day in favour of the CFC-group. Due to a missing SD Scarcella et al. was not included in the meta-analysis.

The last decade clinicians focus on a speedy recovery with fast-track protocols. Amongst the analyzed studies are studies that date back to as far as 1989. Therefore performance bias in this subset of studies is likely and should be interpreted with care. Cryotherapy appears to be a safe mode of treatment, since only 9 of 596 reviewed intervention patient's experienced mild adverse events, which resolved after cessation. Serious complications related to cryotherapy are reported to be 0.0025%[40].

Only 2 of the included articles reported on costs, none of the studies undertook a cost-benefit analysis. Especially in the current times this is vital information for health care decision makers when considering CFC.

One should bear in mind that, although conceived consistent and with care, calculations had to be made to obtain the reported equianalgesic doses since only one of our data requests were followed up upon. This may confound the narcotic analgesic use analysis. Due to the broad application of our intervention of interest heterogeneity is present. The vast majority of the 1,077 study subjects treated with CFC were after knee surgery, with roughly half after

TKA or ACL-reconstruction. A third of the patients were patient's recovering from THA and one study with 40 participants examined CFC after various indications amongst were fractures. We found very different temperature settings applied, however most ranged between 5 and 10°C. The mode of application was fairly consistent, most used continuous application for at least the first 2 days. Only Leegwater et al. and Woolf et al. used the continuous-flow machine on an intermittent basis. Thirty minutes of cryotherapy treatment is sufficient to reduce skin temperature to 10°C easily, analgesic effects takes place between 12°C and 15°C[13,14]. Therefore it seems that analgesic effects can be reached even with this intermittent treatment. Furthermore patients are not bedbound or hindered to attend their physical therapy sessions when treated intermittently.

All applications of CFC where in a (semi) elective setting, we only found one study conducted by Stöckle et al. that evaluated CFC with regard to fracture surgery. Cryotherapy applied to this disease entity might yield more beneficial results due to the duplicate trauma, the fracture itself and the surgical trauma. Cryotherapy treated patients might benefit in a higher degree, due to attenuation of the inflammation reaction that a fracture encompasses. However since no high-quality studies have been performed with regard to the application of CFC in the postoperative recovery of fracture surgery, this should be the focus for future trials.

In conclusion, though continuous-flow cryotherapy use is widespread and considered to have the potential to enhance recovery and exert analgesic effects in sports and clinically, the scientific rationale for its application is lacking. The current evidence is insufficient to make an adequate judgment about whether to advocate cryotherapy or not. It does seem to have mild advantages in regard to postoperative opioid consumption and postoperative blood loss when applied in the recovery phase after elective surgery.

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Chapter 3

Cryocompression therapy after elective athroplasty of the hip

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Abstract

Pneumatic compression and cryotherapy are successfully employed in the management of acute tissue damage. The Game Ready System (GRS) combines cyclic compression and cryotherapy. No randomised controlled trial has been performed that assess the effects of combined cyclic compression and cryotherapy in total hip arthroplasty (THA).

We observed postoperative pain, morphine usage, blood loss, wound discharge, patient and medical staff satisfaction, together with the feasibility of a cryocompression machine, total hospital admission time, infection rate, deep vein thrombosis, and short-term prosthesis related problems in this context. Thirty patients, mean age 68 years (range 31-83 years) undergoing elective hip arthroplasty for end-stage osteoarthritis were included. Control patients (n=15) received a tricot compression bandage alone, and intervention patients received a tricot compression bandage plus intermittent cryocompression therapy 15 times for 30 minutes.

Haemoglobin levels on postoperative day (POD) 1 dropped 2.34 mmol/l in the control group and 1.87 mmol/l in the intervention group (p=0.027). At POD 3 haemoglobin levels were reduced by 2.63 and 2.16 respectively (p=0.646). A trend was observed towards lower morphine usage, shorter hospital admission time and less wound discharge in the study group. No difference was found in postoperative pain scores. One event of deep venous thrombosis occurred in the control group. Intermittent cryocompression therapy therefore appears to reduce postoperative blood loss. A trend towards less analgesic use, shorter hospital stay, less wound discharge and less pain at 6 weeks postoperatively was also observed.

Introduction

Total hip arthroplasty (THA) is one of the most frequently performed and cost-effective operations in orthopaedic surgery (1). The average admission lasts 5 days and overall infection rates range from less than 1 to 3% (2-4). Postoperative pain, wound discharge, haematoma and venous thrombosis can prolong hospital time and delay mobilisation. Postoperative pain is a common problem (5-7), and increases the risk of deep venous thrombosis (DVT) and prolonging hospital stay. The incidence of DVT after THA ranges from 1% to 7%, and early postoperative mobilisation is thought to reduce the risk (8, 9).

Cryotherapy and intermittent pneumatic compression (IPC) can minimise postoperative pain, blood loss, haematoma formation and wound discharge, and improve local blood circulation thus reducing the risk of DVT (10-14). Cryotherapy is employed in the management of acute soft tissue injury, and its effectiveness in preventing swelling as well as exerting analgesic effects is well recognised (12-17).

Hospital admission time can be shortened by using cryotherapy after THA (18), and because of its feasibility and cost-effectiveness standard use has been advocated (19, 20). To our knowledge, no study has been performed in which cyclic cryotherapy and compression therapy have been combined. The Game Ready System® (GRS) hip/ groin-wrap (Almeda/US, Coolsystems Inc.) combines these modalities (Figure 1), in addition to a standard tricot compression bandage (Figure 2). We report a prospective, randomised, pilot study on the effect of intermittent cryocompression therapy following elective THA.

Figure 1. Game Ready hip/groin wrap.

Figure 2. Tricot compression bandage.

Primary outcomes measures were numeric rating scale (NRS) pain scores, analgesic use and wound discharge. In addition, the feasibility of the system was assessed together with patient satisfaction.

Patients and methods

Patients

This pilot study was evaluated and approved by our ethical board committee (DOI 20-09-2010, METC number: 586.10). Between November 2010 and May 2011, all eligible patients undergoing elective THA for end-stage osteoarthritis were asked to participate. Written informed consent was obtained prior to treatment and randomisation. Patients were excluded if their preoperative weight was less than 60 kg, perioperative blood loss of more than 500 ml, blood transfusion was needed during admission, preoperative use of erythropoietin, preoperative osteosynthesis materials were in situ in the operated leg, their American Society Of Anesthesiologists (ASA) score was 3 or 4, they had cryoglobulinaemia, decompensated hypertonia, morphine allergy, angiopathy in the operated leg, a history of deep vein thrombosis, or if they underwent general anaesthesia. Low weight was considered a contraindication due to hospital policy. Blood loss of more than 500 ml can induce vasoconstriction, and combined with cryocompression this can induce ischaemia of the distal extremity. Blood transfusion can confound outcome variables (haemoglobin and wound discharge) through an associated coagulopathy. The use of erythropoietin can confound the postoperative estimation of haemoglobin.

Patients were randomised in two groups, (GRS-intervention and control), 15 patients in each. Randomisation took place on the day of operation prior to surgery with the use of sealed opaque envelopes.

Materials

Total hip arthroplasty was performed using an uncemented Zweymüller prosthesis via a transgluteal approach. All wounds were closed with staples. All patients received a urinary catheter. After surgery blood loss was assessed and the patient was admitted to the orthopaedic ward. Regional anaesthesia consisted of 100-150 mcg morphine administered intradurally. The postoperative analgesic protocol consisted of standard acetaminophen 1000 mg 4 times daily, meloxicam 15 mg once daily and (on request) morphine retard 15 mg twice and morphine solution 2 mg/ml 10 ml six times daily.

After surgery patients were equipped with a patient controlled analgesia (PCA) pump for 2 days, containing 100 mg morphine and 2.5 mg droperidol in a 100 ml saline solution administered intravenously. Pump settings allowed a bolus infusion of 2 ml, lockout-time of 10 minutes, and maximum administration of 24 mg/4 hrs.

Postoperatively, the 15 control patients received an absorbing bandage together with a tricot compression bandage. The intervention group received the same bandage as the control group and GRS. The Game Ready System® hip/groin-wrap was applied over the tricot bandage. Each treatment cycle consisted of 30 minutes of cryotherapy and cyclic compression therapy simultaneously. The minimum non-GRS-treatment interval was 4 hours. The degree of pressure could be adjusted to low (15 mm Hg), medium (50 mm Hg) or high (75 mm Hg). The degree of cooling could be adjusted from 4°C to 12°C. The standard setting used in the study was 4°C, but if this was unacceptable to the patient the temperature or pressure was adjusted accordingly. All patients in the intervention group received the same GRS-treatment schedule: the day of surgery twice on low pressure, first postoperative day medium pressure 4 times, the second postoperative day medium pressure 4 times, third postoperative day high pressure 4 times, the fourth and final treatment day once at high pressure. Given the non-treatment interval, a normal 4 times treatment cycle consisted of: 8:00 h, 12:00 h, 16:30 h and 21:30 h.

Data collection

All patients underwent a preoperative assessment including registration of preoperative age, ASA-classification, body mass index (BMI), Harris hip score, NRS, medication use, blood pressure, heart rate, inner ear temperature and comorbidities. A preoperative blood sample was taken from which haemoglobin, haematocrit, and mean corpuscular volume (MCV) were measured. Each treatment cycle GRS settings, NRS, inner ear temperature, blood pressure, heart rate, and use of medication were recorded before and after treatment, and in control patients recordings were performed once. Haemoglobin, haematocrit and MCV were measured on day 1 and day 3 postoperatively. The postoperative haemoglobin concentrations were subtracted from the preoperative concentrations, and the concentrations on day 3 were subtracted from day 1 concentrations. The dressing was inspected daily and discharge through was recorded (yes or no), and 2 days after surgery the wound was inspected. Urinary catheters were removed and patients commenced physiotherapy on the first postoperative day. On the second postoperative day total morphine usage through the PCA pump was recorded and

the pump was discontinued. All oral administered morphine at the patient's request was noted. All morphine used was recalculated to oramorph. The following proportion was used: oramorph 1: oxycontin 1,5: morphine intravenous (PCA) 3 (21).

At discharge patients were asked to fill in a standardised questionnaire about their experience with the GRS. After discharge all patients were evaluated six weeks. During these assessments NRS, wound discharge, deep or superficial infection, DVT, early septic loosening of the prosthesis and use of analgesic medication were recorded. At the end of the study medical staff were interviewed about their experience with the GRS. All prospective data of the 30 patients were collected using the SPSS program for statistics. (PASW Statistics 18, release 18.0.0, 30 July 2009).

Results

Patients

Of the 30 randomised patients, 4 did not receive the (complete) treatment. One patient in the control group wanted to be allocated to the GRS group, 2 patients in the intervention group had excessive perioperative blood loss, which necessitated exclusion and one patient in the intervention group discontinued GRS use because of discomfort due to inability to urinate. There were no differences between the two groups regarding baseline characteristics (Table 1).

Table 1. Baseline characteristics.

	Cryocompression	**Controls**
Number of cases	15	15
Age (yrs.)	66 (47-82)	68 (31-83)
Sex (m/f)	8/7	4/11
ASA-class	1.67 (1-2)	1.87 (1-3)
BMI	26 (19-33)	27 (19-36)
NRS preoperative	1 (0-2)	1.4 (0-3)

All displayed results were not statistically significant. Values are shown as mean (range). ASA: American Society of Anesthesiologists classification. BMI: Body mass index (kg/m^2).

GRS settings

On POD 1 about half of the patients had their pressure setting reduced. Thereafter 2 patients per day (on average) had their pressure reduced, and cooling was incidentally reduced.

Haemoglobin

No difference in regard to peroperative blood loss was found (Table 2). When compared at POD 1, study group patients had a 0.55 mmol/l (statistically significant) smaller decline in haemoglobin level. At POD 3 this advantage in haemoglobin levels persisted (0.47 mmol/l smaller decline in study group patients) (Table 2).

Morphine usage

When pooled together total oramorph usage in the control group was 100 mg compared to 84.7 mg in the intervention group (Table 2).

Wound discharge

All *'yes'* or *'no'* outcomes of wound discharge were pooled together from the day of surgery until POD 4. Analysis through Mann-Whitney-U test showed lower discharge rates in the intervention group (Table 2).

Total admission time

The mean total hospital stay in the intervention group was 4.75 compared to 5.0 in the control group (not significant) (Table 2). The relatively large SD originating from the control group was attributed to the 10-day hospital stay of 1 patient in contrast to the average 5-day stay. There were no differences relating to reinsertion of the urinary catheter or earlier physiotherapy between the 2 groups.

Table 2. Result of outcome variables.

	Cryocompression (n=12)	**Controls** (n=14)	**Significance**	**Test**
Blood loss peroperative (ml)	280 (63)	256 (129)	0.616	Levene
Haemoglobin OK+1 – preop	-1.79 (0.73)	-2.34 (0.39)	0.027	Levene
Haemoglobin OK+3 – preop	-2.16 (1.0)	-2.63 (0.49)	0.167	Levene
Haemoglobin OK+3 – OK+1	-0.38 (0.53)	-0.29 (0.36)	0.646	Levene
Wound discharge	7.13	12.09	0.053	Mann-Whitney
Total oramorph usage	84.7 (43.6)	100 (73.5)	0.593	Levene
Days of leakage	1.83 (2.34)	2.92 (3.23)	0.349	Levene
Total admittance time (days)	4.75 (0.75)	5.00 (1.63)	0.633	Levene

Values are shown as mean (SD) unless otherwise specified.

Questionnaires

When questionnaires were analysed patients were generally positive about cryocompression treatment. Pain experienced was less and mobilisation was faster. A

number of patients had their temperature setting adjusted to a less cold setting, and high pressure was considered to be more comfortable. When asked, they would recommend cryocompression to other patients (Table 3).

Medical staff was positive about GRS use. Applying the GR-wrap was generally easy, except on the day of surgery when patients' legs were still partially sedated, and on the first POD because of postoperative pain. Some difficulty occurred with the GRS in obtaining enough ice cubes, especially when multiple patients were treated simultaneously.

Complications

At 6 weeks follow-up one patient from the control group had developed deep vein thrombosis despite prophylactic treatment. No infections, use of antibiotics or early septic loosening had occurred at 6-week follow-up. There were no deaths or other adverse events in the intervention or control group.

Table 3. Patient questionnaire Game Ready experience.

	Statement	Average score
1.	I experienced the use of GRS as pleasant	0.54
2.	I had less pain during use of GRS	0.67
3.	I had the temperature adjusted (yes/no)	10% yes
4.	I liked the coldest setting best	-0.11
5.	I had the pressure setting adjusted (yes/no)	45% yes
6.	I liked the highest pressure setting best	0.10
7.	I applied the GRS-wrap myself (yes/no)	100% no
8.	Applying the GRS-wrap was easy to perform	0.56
9.	I kept the wrap on, even between treatments	-1.1
10.	I was treated on time	0.90
11.	Nurses had to come by often because of GRS	-1.2
12.	I had little discomfort from GRS treatment	0.81
13.	I think GRS is a good treatment	0.90
14.	I do not regret use of GRS	1.20
15.	I think I recovered faster because of GRS	0.70
16.	I would recommend GRS to other patients	0.90
17.	I would have like to be treated more and longer with GRS	-0.60

Number of respondents = 17. GRS: Game Ready System.

Discussion

Our study has demonstrated a lesser decline in postoperative blood loss in patients using cryocompression therapy. In 2005 Johansson et al. used a pneumatic compression bandage in 51 patients; the 54 control patients were given a wound drain. They found that the necessity for transfusion was the same, but the number of transfused units and wound discharge was significantly less in the compression group (13). Hörnberg et al in 2002 found that patients who were given a standard dressing over a compression dressing required significantly more blood transfusions (22). Fujisawa et al. encountered a significant reduction in leg circumference when calf-thigh IPC was applied compared to plantar IPC after THA, reflecting reduced wound oedema and blood loss (11). Our findings similarly suggest a trend towards less wound discharge in the cryocompression therapy group. Liu et al. observed that compression caused shear stress of the vascular endothelium and therefore vasodilatation, resulting in reduced swelling (10). We did not measure leg circumference, but the reduced decline of haemoglobin levels indicated a reduced peripheral leakage of blood and possibly oedema fluid. Reduction in leg swelling and shear stress induced vasodilatation prevent haematoma and stasis, both of which are risk factors for infection.

It not only reduces postoperative leg swelling, but pneumatic compression also reduces thrombogenesis in the early postoperative period (11, 23, 24). Currently, wound drains are widely employed to minimise wound discharge, infection and haematoma, but such benefits have never been statistically proven (25-29). A recent systematic review showed no significant difference in the incidence of wound complications with or without drains, but the necessity for transfusion was higher in when drains are used (30).

A trend towards lower morphine use was observed in our study, but statistical significance was not achieved, although others have demonstrated a significant difference in opiate use (12), and analgesic requirements and pain scores (19, 31). The reduced efficacy of cryotherapy after THA when compared to knee arthroplasty may result from the inability of the therapy the reach deeper layers effectively (32). Although it is reported that patients start mobilisation faster, this was not seen in our study, probably due to our protocol, in which intervention patients and controls alike received physiotherapy on the first postoperative day and were discharged on POD 4.

Two adverse events occurred; deep vein thrombosis in a control patient and inability to urinate in one intervention patient. The cryocompression bandage partially covers the

pubic area thus this adverse event can be a side effect of cryocompression treatment. After this event, we covered the pubic area with an extra towel before applying the GRS. Our pilot study conclusions are hampered by a small sample size. However the advantage of a cryocompression in postoperative blood loss reduction appears to be clear. In regard to wound discharge scoring, a significant interobserver bias may have occurred, because the ward nurse or doctor who assessed the issue changed from day to day. Supplying the GRS with sufficient ice cubes was difficult at the start because of insufficient capacity of the ice cube machines. The manufacturer supplied an additional machine and a refrigerator for storage capabilities, and this provided sufficient capacity for multiple treatments simultaneously.

During the evening shift ward nurses found it difficult to apply the GRS in time (or at all) because of limited personnel. To overcome this the treatment cycles might be adjusted to 8:00 h, 12:00 h, 16:00 h, and 20:00 h. Patients were pleased with the cryocompression bandage, and reported it to enhance comfort and reduce wound pain, but as they were treated on the same ward as the control patients bias cannot be ruled out.

With a reduction in blood loss caused by cryocompression blood transfusion can be averted in the acute postoperative phase of THA. There might also be lower postoperative morphine consumption. Cryocompression reduces stasis and haematoma formation, and may therefore reduce the risk of infection. However, our sample size was too small to demonstrate advantages in wound discharge rates, hospital admission time and infection rates. A larger trial may help to address these matters.

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Chapter 4

The efficacy of continuous-flow cryo and cyclic compression therapy after hip facture surgery on postoperative pain: design of a prospective, open-label, parallel, multicenter, randomized cotrolled, clinical trial

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Abstract

Background: The number of hip fractures and resulting post-surgical outcome are a major public health concern and incidence is expected to increase significantly. The acute recovery phase after hip fracture surgery in elderly patients is often complicated by severe pain, high morphine consumption, perioperative blood loss with subsequent transfusion and delirium. Postoperative continuous-flow cryocompression therapy is suggested to minimize these complications and to attenuate the inflammatory reaction that the fracture and subsequent surgical trauma encompass. Based on a pilot study in patients undergoing total hip arthroplasty for osteoarthritis, it is anticipated that patients treated with continuous-flow cryocompression therapy will have less pain, less morphine consumption and lower decrease of postoperative hemoglobin levels. These factors are associated with a shorter hospital stay and better long-term (functional) outcome.

Methods/Design: One hundred and sixty patients with an intra or extracapsular hip fracture scheduled for internal fixation (intramedullary hip nail, dynamic hip screw or cannulated screws) or (hemi) arthroplasty will be included in this prospective, open-label, parallel, multicenter, randomized controlled, clinical superiority trial. Patients will be allocated to two treatment arms: group 'A' will be treated with continuous-flow cryocompression therapy and compared to group 'B' that will receive standard care. Routine use of drains and/or compressive bandages is allowed in both groups. The primary objective of this study is to compare acute pain the first 72 hours postoperative, measured by numeric rating scale for pain. Secondary objectives are: (non-) morphine analgesic use; adjusted postoperative hemoglobin level; transfusion incidence; incidence, duration and severity of delirium and use of psychotropic medication; length of stay; location and duration of rehabilitation; functional outcome; short-term patient-reported health outcome; general and cryotherapy related complications and feasibility.

Background

Hip fractures are one of the most important causes of long-term disability and a significant public health issue[1]. Incidence varies on a global scale[2, 3], but is estimated to vary between 414 and 957 per 100,000 in the USA[4]. Due to aging of the population in general a significant increase in hip fracture numbers is expected[5].

A hip fracture is a serious condition associated with high morbidity, one-year mortality rates up to 29%[2, 4], severe pain[6, 7] and significant decline in functional status[8-11]. Less than 50% of (semi-) independent living elders return to their pre-fracture habitat[11, 12]. Amongst the most serious of complications is the onset of a delirium, which has a particular high incidence of 45% in hip fracture populations[13-15]. Possibly because this condition is known to be extremely painful[15-18], and patients with pain are nine times more likely to develop a delirium[18-20] and furthermore take longer to ambulate and consequently, have longer length of stay[9].

Upon admission 40% of hip fracture patients is anemic[21] and observed intraoperative blood loss is systematically underestimated; up to six-fold in excess in extracapsular fractures[22]. The continued postoperative decline in hemoglobin levels results from, among others, surgical site bleeding[22]. This yields a specifically high transfusion rate in this frail elderly patient category where anticoagulant or thrombocyte aggregation inhibitors use is relatively prevalent[23]. Besides (postoperative) surgical site bleeding, the fracture trauma and subsequent surgical stabilization procedure induce an inflammatory response causing leakage of plasma proteins and migration of inflammatory cells that lead to local peripheral vasodilatation and increased capillary permeability, ultimately causing edema[24]. Edema gradually develops during the first week post surgery, is more pronounced in intertrochanteric fractures and is correlated with reduced knee extension strength and functional performance[25, 26]. In an attempt to reduce edema and postoperative hemorrhaging compressive wound dressings are applied[27-29]. Dynamic pneumatic compressive devices not only reduce blood loss, edema and offer a better (hemo) dynamic profile in the deep venous and lymph system, it also exerts analgesic effects and reduces the inflammatory response when combined with a cryotherapy adjunct[30-35].

In a pilot study on 30 patients in a Dutch teaching hospital an apparatus with continuous-flow cryotherapy combined with intermittent compression was used in the postoperative setting of hip arthroplasty for end-stage osteoarthritis[36]. A trend towards lower visual analogue scale (VAS) pain scores and less morphine use was observed, and patients receiving continuous-flow cryocompression (CFC) therapy had statistical significant less decline in postoperative hemoglobin levels. In two randomized controlled trials evaluating continuous-

flow cryotherapy (without compression adjunct) in 45 total hip[37] and 208 total hip and knee arthroplasty[38] patients, lower pain scores were observed and less morphine was used. Furthermore length of stay was 1.4 days shorter when continuous-flow cryotherapy was applied after hip arthroplasty in 74 patients[39].

Currently all published trials studying CFC therapy focus on semi-elective procedures such as anterior cruciate ligament reconstruction and total knee arthroplasties[40]. To our knowledge, no randomized controlled trial exists evaluating the efficacy of CFC therapy in the acute postoperative recovery phase of hip fracture patients[40]. As hip fracture patients have duplicate trauma, severe pain, fracture site bleeding with related inflammation and associated soft tissue damage these patients are expected to benefit most from CFC therapy.

Aim

The aim of the current study is to evaluate the efficacy of CFC therapy on pain in the first 72 postoperative hours (h) of hip fracture patients. The secondary aim is to evaluate the effects on (non-) morphine analgesic use; postoperative hemoglobin level; transfusion incidence; delirium incidence and severity; use of psychotropic medication; length of stay; short-term location and duration of rehabilitation; functional outcome; short-term patient-reported health outcome; general and cryotherapy related complications. Furthermore the feasibility of a cryocompression device on orthopedic wards is assessed.

We hypothesized that 1) CFC therapy will lower perceived pain levels and morphine consumption; and 2) will reduce postoperative blood loss and transfusion incidence; and 3) reduced pain by CFC therapy will lead to lower delirium incidence and 4) enhance functional recovery, leading to shorter length of stay in postoperative hip facture patients.

Methods

Study design

This study is designed as a prospective, open-label, parallel, multicenter, 1:1 randomized controlled, clinical superiority trial in accordance with CONSORT and SPIRIT guidelines[41, 42]. Eight orthopedic, surgery and/or geriatric departments of four middle sized teaching hospitals and one academic hospital in the Netherlands will participate: The Spaarne Hospital, Hoofddorp; Medical Center Alkmaar, Alkmaar; Amstelland Hospital, Amstelveen; Kennemer Gasthuis hospital, Haarlem; VU University Medical Center, Amsterdam. Participant criteria are displayed in table 1.

Table 1. Criteria for participants in the trial.

Inclusion criteria	Exclusion criteria
* Intra or extra capsular proximal femur fracture	* Fractures at multiple foci
* Aged 18 years and over	* Open fracture/wounds[§]
* Informed consent or proxy consent	* Acetabular fracture
	* (Suspicion of) concomitant malignancy
	* BMI > 40
	* Preoperative osteosynthesis materials in situ in the ipsilateral leg above knee level
	* Unwilling to give proxy consent
	* Morphine allergy or dependence
	* ASA ≥ 4
	* Cryoglobulinemia
	* M. Raynaud
	* Central neuromuscular disorder
	* Absent ADP/ATP pulsations in the injured extremity
	* History of deep vein thrombosis
	* Patient delay > 24 hours
	* NYHA-class ≥ 3
	* IQ-CODE score ≥ 4.6
	* Long-acting femoral blocks

a Open wounds unable to close per primam, * The IQ-CODE is only administered if the clinician has doubts about the cognitive status of the intented participant. BMI: body mass index, ASA: American Society of Anesthesiologists, ADP dorsal pedal artery, ATP posterior tibial artery, NYHA New York Heart Association, IQ-CODE Informant Questionnaire for the Cognitive Decline in the Elderly, LIA local infiltration anesthesia, HD hemodynamic.

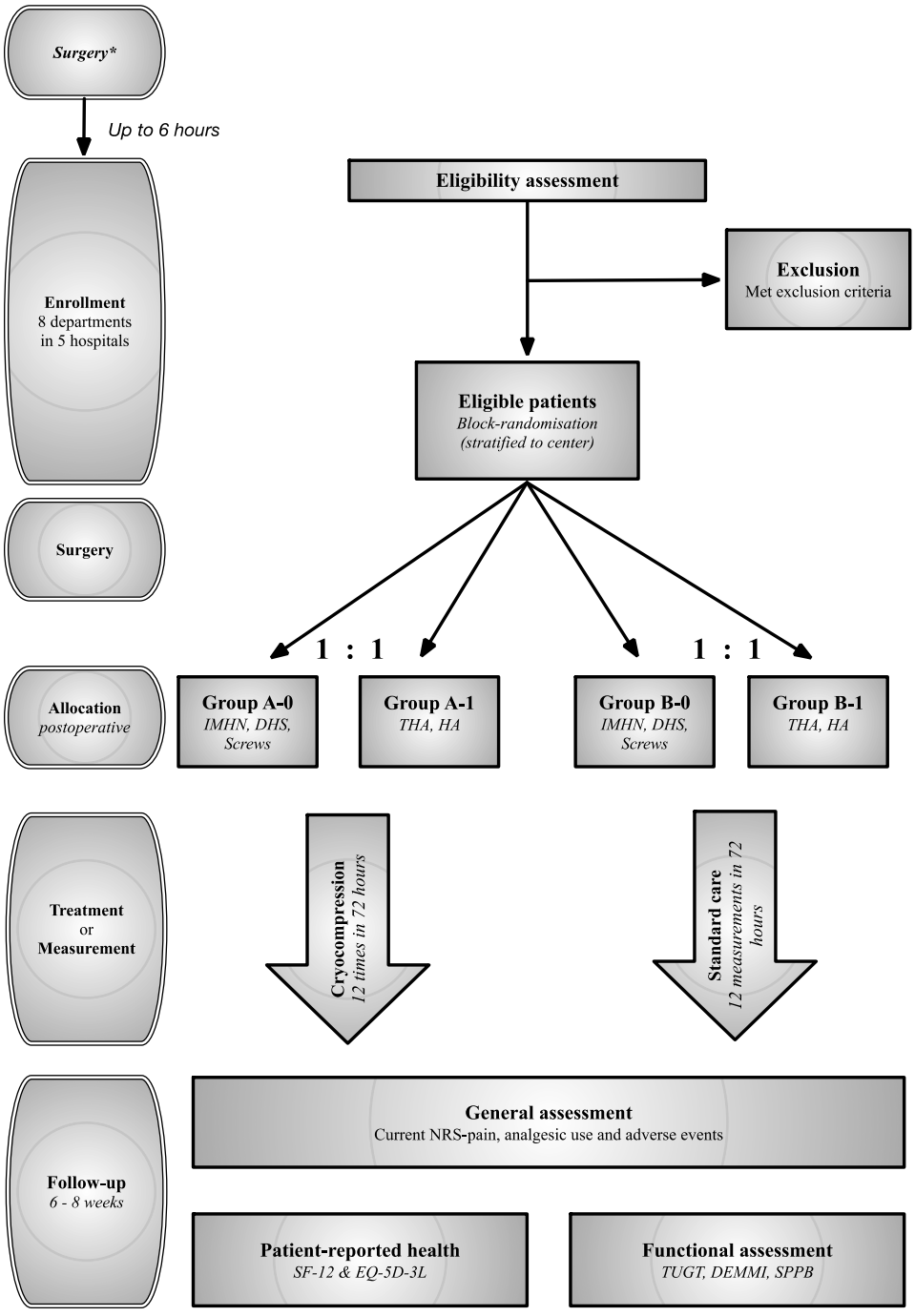
Randomization

A balanced 1:1 block (size six) randomization stratified by center will be performed directly after surgery, using Research Manager (RM; version 4.5.0.1), a web-based computer program (Figure 1). A research nurse who is not involved in the study generates the allocation sequence with Research Manager. Intramedullary hip nail (IMHN), dynamic hip screw (DHS) and cannulated screws are grouped for block randomization, as are total hip arthroplasty (THA) and hemiarthroplasty (HA). The intervention group 'A' will receive CFC therapy postoperative; the control group 'B' will receive standard care. Due to the nature of the study no blinding can exist as patients, family and physicians notice the use and settings of CFC therapy. Assessing physicians randomize participants with a digital logon to the web-based system or the physician can contact the coordinating investigator and he will randomize the participant.

Standard care

On arrival at the A&E department patients receive acetaminophen intravenously, diclofenac (if not contraindicated) and morphine subcutaneously or fentanyl intravenously until numerical rating scale (NRS)-pain scores have dropped below 4. Upon admission the local protocol is started (Table 2). At all centers the "as needed" medication is administered when NRS-pain score is 4 or higher and no excessive (opioid-induced) sedation is present. Patients who are deemed able are given intravenous patient controlled analgesia (PCA) pumps with morphine (Table 2). In patients older than 70 years the bolus setting is reduced by 50%. Preferably patients are operated on using spinal anesthesia. In order to put the patient in an upright position for spinal anesthesia administration femoral blocking with short-acting analgesics can be used. All centers use bupivacaine 0.5% between 2.0 ml to 3.0 ml administered at the lumbar level. Additional (long acting) analgesics administered during surgery are noted. The participating centers adhere to the Dutch national guidelines for surgical technique for the various fracture types[43]. Postoperatively, on the day of surgery or the first postoperative day physical therapy is commenced once or twice daily. Physical therapy sessions are usually 30 minutes long. In the period in which the focus of this study lies physical therapy focuses on strengthening of quadriceps and gluteal musculature, walking and making transfers.

Figure 1. Flowchart GRAPES-trial.



* Patient enrollment is allowed up to 6 hours postoperative. IMHN: intramedullary hip nail; DHS: Dynamic Hip Screw; screws: cannulated screws; THA: Total Hip Arthroplasty; HA: Hemiarthroplasty; SF-12: short form-12; EQ-5D-3L: EuroQol; TUGT: timed up and go test; DEMMI: de Morton mobility index; SPPB: short physical performance battery.

Table 2. Hospital protocols.

		Analgesic protocol				
Hospital	**Thrombosis prophylaxis**	**Standard**		**As needed**		
Spaarne Hospital	Fraxiparine 2850IE daily‡	Acetaminophen 1000mg 4x daily	Diclofenac 50mg 3x daily*	Oramorph 10mg 3x daily	*or*	MS-contin 10mg 2x daily *and* Oramorph 10mg 6x daily or sc morphine 10mg 6x daily
VU University medical centre and Medical Center Alkmaar	Fraxiparine 2850IE daily‡	Acetaminophen 1000mg 4x daily	Diclofenac 50mg 3x daily*	Piritramide 10mg 6x daily		
Kennemer Gasthuis Hospital	Fraxiparine 2850IE daily‡	Acetaminophen 1000mg 4x daily	Diclofenac 50mg 3x daily *or* 75mg iv 2x*	Oxycodone 5 or 10mg 6x daily	*or*	Fentanyl titration
Amstelland Hospital	Enoxaparine 40mg daily	Acetaminophen 1000mg 3x daily	Diclofenac 75mg im/iv 2x daily*	PCA-IV morphine *If considered able*	*or*	Tramadol 50mg 3x daily *or* Piritramide 10mg 6x daily

Reported dosages are dosages given in 24 h. im: intramuscular, iv: intravenous, sc: subcutaneous, PCA patient controlled analgesia. a Dosage is doubled in patients weighing > 80 kg. b If no contraindications exist. c Dosage is reduced by 50 % in patients aged ≥ 70 years.

Study apparatus and treatment schedule

Continuous-flow cryocompression therapy is applied by using the 'Game Ready System' (GRS; CoolSystems Alameda, California). Through an anatomically designed hip/groin wrap covering most of the thigh and pelvis up to the iliac crest, the GRS simultaneously delivers both adjustable continuous-flow cryotherapy and intermittent compression through a portable control unit filled with ice and water. The machine has four pressure settings: no pressure, low pressure (5-15 mm Hg), medium pressure (5-50 mm Hg) and high pressure (5-75 mm Hg). Temperature can be adjusted between 4°C and 13°C and is indicated by one, two or three snowflakes. The lowest temperature is started and maintained throughout the study if feasible. Pressure is started at '*low'* and is increased one step per 4 treatments (Table 3). Depending on the end of surgery patients will be categorized in three treatment schedules with respective start and end times (Table 3). If patients are uncomfortable the appropriate adjunct will be adjusted stepwise until a comfortable setting is reached, deviations are noted. Adjustments are recorded and comfortable settings are maintained according to the discomfort flowchart (Figure 2). Patients will be treated between 10 to 12 times during the first 72 postoperative hours, each cycle lasting 30 minutes. Preferably, treatment cycles and control measurements during the first 72 postoperative hours are performed at fixed moments: 8:00 h, 12:00 h, 16:30 h, and 21:30 h. The GRS wrap is only in place when CFC therapy is administered and applied/removed by the nurse.

Co-interventions

In case of discomfort the appropriate adjunct is adjusted accordingly (Figure 2) and supplemental analgesics are given as needed (Table 2). In both groups no restrictions are made towards regularly used static compressive bandages and/or wound drains (with or without autologous re-infusion). Geriatricians are consulted in a standard fashion in patients aged 70 years and over.

Admission

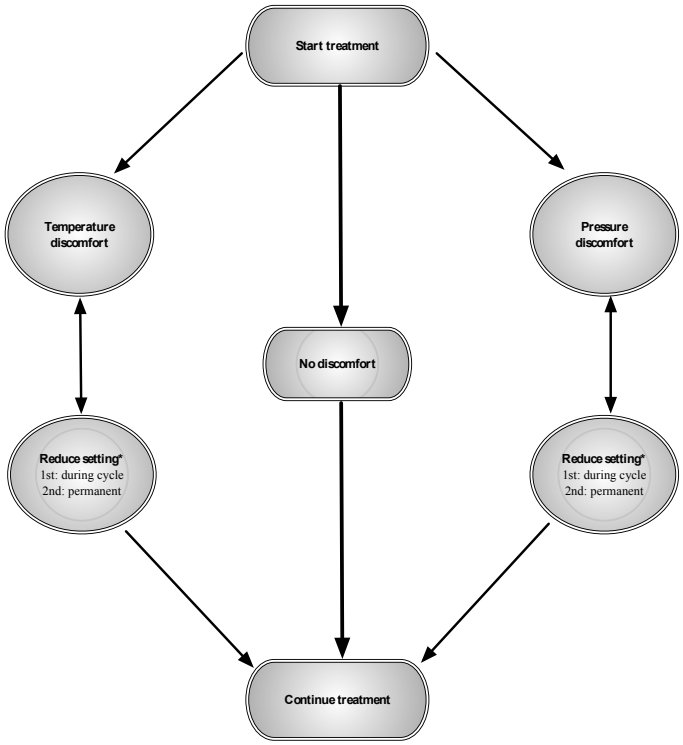
Patient demographic data are noted on admission as well as American Society of Anesthesiologists (ASA) class, current NRS-pain, delirium observation screening (DOS)-score, activities of daily living (ADL)-Katz score and the New Mobility Score (NMS)[44-46]. Time of injury and arrival at the A&E department is noted. Patient delay intervals longer than 6 hours are actively explored and reasons for delay are clarified e.g. if a patient was prone and unable to call emergency services.

A standard preoperative venous blood sample is taken in which hemoglobin, haematocrit and mean corpuscular volumes values are measured. Upon admission the local hospital analgesic and antithrombotic protocol is started (Table 2). A geriatrician is consulted preoperatively in patients who are older than 70 years and will focus on: polypharmacy, risk reduction of falls, prevention and/or treatment of delirium, optimization of nutritional status. The ADL-Katz score and delirium risk assessment score (DRAS)[47] are completed upon admission; furthermore precipitating factors for delirium (bladder infection, retention, fixation, sleep deprivation, electrolyte abnormalities including renal function impairment) are noted throughout the hospital stay. After the operation the surgeon notes the intraoperative blood loss, the type of implant, if applicable intraoperative complications, randomizes the patient and determines the patient's treatment schedule.

Cryocompression therapy and clinical measurements

Intervention patients start with CFC therapy 6 hours postoperative, until 72 hours postoperatively (Table 3). A trained ward nurse will conduct the pain assessments when the patient is in bed or stationary in a chair for at least five minutes. Directly before and directly after CFC therapy vital signs including NRS-pain are measured by the ward nurse, when the patient is prone in bed for at least five minutes before commencing and five minutes after cessation of CFC therapy. In control patients only NRS-pain is measured at the same time as intervention patients would normally be assessed. All pain measurements are continued throughout the first postoperative 72 hours for control and intervention patients alike. Three times daily DOS-scores[48] will be obtained; in case of a DOS-score higher or equal to three, geriatricians diagnose and subsequently monitor delirium severity with the delirium rating scale revised 1998 (DRS-R-98)[49] on a daily basis or until the score drops below 12.25 out of 39. Time and amount of administered (psychotropic) medication will be documented. The nurse and ward doctor inspect the wound and dressing in accordance with routine care. Blood samples are taken at postoperative day one and three, and post transfusion if applicable. The timed up and go test (TUGT) is performed before discharge. The TUGT will be administered in all patients who are able. If no weight bearing is allowed or patients are physically unable, than the test will be postponed to the outpatient visit.

Figure 2. Discomfort flowchart.



*: If a patient reports discomfort for the second time at the same setting the setting is permanently reduced to the setting deemed comfortable.

Table 3. Treatment schedule and pressure settings.

End time surgery	Schedule	Start time
10:00 – 16:00h	*A*	16:30h
16:00 – 21:00h	*B*	21:30h
> 21:00	*C*	8:00h

Cycle no. →	1	2	3	4	5	6	7	8	9	10	11	12
OK+0	*A:* – Low	*A* – Low										
	B: – Low	-										
	-	-										
OK+1	-	-	*A* – Low	*A* – Low	*A* – Med	*A* – Med						
	-	*B* – Low	*B* – Low	*B* – Low	*B* – Med	-						
	C – Low	*C* – Low	C – Low	*C* – Low	-	-						
OK+2					-	-	*A* – Med	*A* – Med	*A* – High	*A* – High		
					-	*B* – Med	*B* – Med	*B* – Med	*B* – High	-		
					C – Med	*C* – Med	*C* – Med	*C* – Med	-	-		
OK+3									-	-	*A* – High	*A* – High
									-	*B* – High	*B* – High	*B* – High
									C – High	*C* – High	*C* – High	*C* – High

Regular treatment times: 8:00 h; 12:00 h; 16:30 h; 21:30 h.

The MMSE is administered for data stratification purposes[50-52], if patients are delirious during admission the MMSE is postponed to the outpatient visit.

In order to document experiences with CFC therapy all treatment patients will fill in a questionnaire at discharge, which is specifically drafted for this study. During the study a booklet will be made available into which staff can write down prevalent occurring technical GRS-related problems.

Outpatient visit

At the single outpatient study visit between six to eight weeks, the following parameters are assessed; current NRS-pain; analgesic use; mobility status by the TUGT, de Morton Mobility Index (DEMMI) and the short physical performance battery (SPPB); current living situation (rehabilitation or at home); wound status and complications (thrombosis, consolidation, infection, readmission). Furthermore the self-assessment health questionnaires EuroQol(EQ)-5D-3L and Short Form (SF)-12 are completed. If not administered during admission the MMSE is completed.

Data collection and handling

Upon inclusion patient characteristics are entered in RM and postoperative randomization takes place. A unique patient number is generated by RM, which will be used for further data collection on preformatted case report forms (CRF's). Only the coordinating investigator has access to the key-file that can identify patients from RM-generated patient numbers, it will be stored at the coordinating center throughout and after completion of the trial and will not be shared. The Spaarne Hospital is the unrestricted owner of the final dataset; no contractual agreements exist in regard to publication policies. No interim analysis will be performed.

Data management team

The coordinating center comprises of: the principal investigator, the coordinating investigator, a research nurse, a research assistant, a clinical epidemiologist and statistician. The research assistant enters data in RM. The research nurse can be contacted via telephone to solve any (acute) problems that might occur and enters data in RM. The epidemiologist and statistician assist with data analyses and drafting of the manuscript. During the enrolment phase of the trial the coordinating investigator meets bimonthly with the individual principal investigators of the respective centers to assess the current inclusion rate, data quality, protocol adherence and prevalent occurring problems. During enrolment two meetings are planned to collectively

assess problems and deliberate on how to instigate solutions to these problems. All participating personnel are updated on a monthly basis via a newsletter in which current inclusion rates are presented, and to point out points of attention and solutions to frequently observed problems. During physical visits CRF's are collected, assessed for completeness and missing data is retrieved from patient medical records where possible; secondly the appropriateness of administered treatments and (functional) tests is assessed.

Data monitoring committee

Two independent data monitors are instigated and will monitor the trial at two of the five sites where highest inclusion rates are expected. Results of the monitor will be discussed in a committee independently of the investigators and the investigators will be informed both orally and in writing. Since the overall risk of participation in the trial is considered low by the REC/IRB no external safety overseers are required by national guidelines. If selected, external auditing is possible.

Outcome parameters

NRS-pain (primary)

The verbally administered 11-point (range 0-10) NRS-pain is a widely accepted assessment tool and has been validated in many different conditions, amongst is acute pain in the emergency department[53-55]. Reliability and validity has been thoroughly studied in (cognitively impaired) elderly and has shown to maintain acceptable clinometric characteristics[56-58]. NRS-pain scores are compared within the intervention group i.e. pre and post treatment and compared between the intervention and control groups. Cumulated pain scores will be calculated for both groups by adding the (post treatment) pain scores of the first 72 postoperative hours.

Analgesic use

All postoperative oral and parenteral administered narcotic analgesics will be converted and added up using accepted algorithms[59, 60] and compared after the last treatment day, at discharge and at the outpatient clinic. Total milligrams of acetaminophen and non-steroidal anti-inflammatory drugs (NSAID's) are reported in the same fashion. At the outpatient visit patients will be asked about their current pain medication use.

Postoperative blood loss and transfusion incidence

The hemoglobin values from the obtained blood samples at postoperative day one and three will separately be detracted from the preoperative concentration and postoperative day three will be detracted from postoperative day one. An adjustment will be made for the intraoperative blood loss. Indications for transfusion are managed according to the Dutch national guideline[61]. Normovolemic ASA-one patients older than 60 years who lose blood at a single locus will be transfused at 5 mmol/l (8 g/dl). Patients unable to elevate cardiac output for hemodilution are transfused at 6 mmol/l (9.6 g/dl). Incidence of erythrocyte transfusion is noted and compared between groups.

Postoperative delirium

The DRS-R-98 is considered to be a valid and reliable instrument for delirium diagnosis and documenting delirium severity[49, 62]. It takes a trained nurse or physician five to ten minutes to assess the patient and comprise the DRS-R-98 score, and is ideal for longitudinal delirium follow-up. The sensitivity and specificity of the scale are reported to be 92% and 86% respectively. DRS-R-98 scores are compared within and between groups, and newly started psychotropic medication is compared.

Length of stay

Admission time is measured and expressed in two ways: from arrival time at the A&E department until discharge and from end of surgery to until discharge. The discharge criteria in all participating centers are: NRS-pain score below 5 without need for parenteral analgesics and an Elderly Mobility Scale (EMS) score 14 out of 20 or higher[63, 64]. If this some items were deemed unobtainable EMS scores were completed until transfer to a nursing home was arranged. If family members are able to foresee in certain home care aspects the patient could be discharged home. The fulfillment of this care by family is noted and corrected for afterwards.

Functional outcome

Timed up and go test

The TUGT is a functional mobility test that measures the time it takes a patient to get up out of a chair, walk 3 meters and return in a sitting position. The test is easy to use and has sufficient clinometric characteristics[65], can predict short-term risk of new falls[66] and functional outcome over time[67]. Detailed instructions are provided to the patient: rise from the chair (knees at 90° flexion) when you hear *"go"*, walk at safe speed to the mark on the floor

and back, the time stops when you hit the chair with your buttocks. The physical therapist will demonstrate how to perform the TUGT once. The average of three tests will be the final TUGT outcome and no trial run is performed. Postoperative mobilization policy is noted and patients are stratified in three groups: no weight bearing, partial weight bearing or full weight bearing. Preferably no walking aid will be used during the test but if needed, the patient can use any walking aid available, the type of aid used will be recorded. The updated NMS is administered at inclusion for data stratification purposes[44, 45, 68]. NMS will be classified as low when NMS is 2-6 and scored high when NMS is 7-9[46].

De Morton mobility index

The DEMMI is a measure of mobility that has been validated in hip fracture patients[69-71]. The DEMMI is administered by physician or physical therapist observation of the test subject' physical performance, measured in 15 hierarchical domains (three bed, three chair, four static balance, two walking and three dynamic balance items), each measured on a two (able/unable) or three (able/ partial/unable) point scale. It takes a trained person ten minutes to administer the test in elderly patients. The raw score is converted to an interval score.

Short physical performance battery

The SPPB is composed of three tasks: a hierarchical balance task, a short walk at normal speed, and five repetitive chair stands. Low scores in the SPPB have predictive value for a wide range of health outcomes: mobility loss, disability, hospitalization, length of hospital stay, nursing home admission, and death[72-75]. Normative values of SPPB have been published for representative populations by five-year age groups and sex[73]. The strong and consistent association with health status measures demonstrated the validity of the SPPB[76].

Patient reported outcome measures

EQ-5D-3L

The EuroQol/EQ-5D-3L is a validated, generalized and standardized instrument comprising a VAS measuring self-rated health and a health status instrument, consisting of a three-level response (no problems, some problems and extreme problems) for five domains related to daily activities; (i) mobility, (ii) self-care, (iii) usual activities, (iv) pain and discomfort and (v) anxiety and depression. Responses to the health status classification system are converted into an overall score using a published utility algorithm for the Dutch population[77]. A

respondent's EQ-VAS gives self-rated health on a scale where the endpoints are labeled 'best imaginable health state' (100) and 'worst imaginable health state' (0).

Short Form-12

The SF-12 is the abbreviated version of the original SF-36 and scores quality of life in two domains, physical and mental health. It has shown good correlation with the SF-36 and proved valid in many conditions, amongst is orthopedic surgery[78].

Complications

The surgeon monitors all complications that may occur throughout admission, in general or cryotherapy related. After discharge the coordinating investigator verifies if all adverse events are registered and recorded on the appropriate case report form. In case of serious adverse events relating to the treatment patients will no longer receive treatment but will remain in the study for follow-up, if feasible. Severe leakage i.e. multiple bandage swaps daily is noted and registered as an adverse event. Furthermore an intragroup analysis of vital signs is made to determine possible central effects of the administered therapy. Finally, at the outpatient visit patients are assessed for adverse events that may have occurred during rehabilitation. Additional to the EQ-5D-3L and SF-12 questionnaires, intervention patients are asked about their experiences with CFC therapy (Table 5). An additional insurance is taken out to financially compensate participants if applicable. Adverse events are tabulated and reported in the final manuscript.

Feasibility

At the end of the study nursing staff will complete questionnaires specifically drafted for this study (Table 6) and the booklet of prevalent technical problems will be evaluated. A close out visit will be planned in which recommendations for further improvement of the machine will be discussed. Treatment failure i.e. discontinuation of treatment is reported and provided with reasons e.g. discomfort.

Table 4. Outcome assessment points.

		Assessment point						
	Data collection instrument (unit)	**Baseline (ED-department)**	**Day of surgery**	**24hrs**	**48hrs**	**72hrs**	**Discharge**	**6 weeks (outpatient clinic visit)**
Demographics	-	X						
Pre-fracture functional status	NMS	X						X
Delirium risk assessment	DRAS	X						
Cognitive function	IQCODE-N	X						
	MMSE						X	
Blood loss	Intraoperative loss (cc's)		X					
	Drain output* (cc's)			X	X	X		
Outcome								
Pain	NRS	X		X	X	X	X	X
Analgesics	Acetaminophen	X					X	
	NSAID's	X					X	
	Morphine	X					X	
Blood loss	Haemoglobin (mmol/L)	X		X		X		
Transfusion incidence	(Number of PC's)						X	X
Delirium	DOS-score	X	X	X	X	X	X	
	DRS-R-98	X	X	X	X	X	X	
Length of stay‡	(hours)						X	
Functional outcome	TUGT						X	
	DEMMI							X
	SPPB							X
	Rehabilitation location						X	
	Rehabilitation duration						X	
PROM	EQ-5D							X
	SF-12							X
	Satisfaction questionnaire						X	
Complications	General						X	X
	Therapy related						X	X
Feasibility	Questionnaire						At discharge of last patient	

* Including autologous reinfusion. ‡ Start count at admission and at the end of surgery. ±: administered when DOS ≥ 3. ED: emergency department; NMS: New Mobility Score; IQCODE-N: Informant Questionnaire on the Cognitive Decline in Elderly patients; MMSE: Mini Mental State Exam; NRS: Numeric Rating Scale; PC: packed cells; DOS: Delirium Observation Screening; DRS-R-98: Delirium Rating Scale Revised 98; TUGT: Timed Up and Go Test; DEMMI: De Morton Mobility Index; EQ-5D-3L: EuroQol questionnaire; SF-12; Short Form-12 questionnaire.

Table 5. Patient questionnaire.

	Question	Answer options
1.	Did you feel that the pain reduced when treated with cryocompression therapy?	A B C D E
2.	Would you rather have cryocompression treatment than analgesic pain treatment?	A B C D E
3.	The standard setting used was the coldest; did you like this temperature setting?	A B C D E
4.	Did you request the temperature setting to be upped?	Yes / No
5.	Did you like the dynamic pressure adjunct?	A B C D E
6.	The pressure adjunct was elevated every 4 treatments, was the pace to fast?	A B C D E
7.	After treatment the muscles are cooled, did this hinder you in moving around outside of bed?	A B C D E
8.	Would you have liked to be treated more often per day than 4 times?	A B C D E
9.	Would you have liked to be treated longer than 30 minutes per cycle?	A B C D E
10.	Would you have liked to be treated longer than the first 72 hours postoperative?	A B C D E
11.	Did you feel like you recovered faster with cryocompression therapy?	A B C D E
12.	Would you recommend the use of cryocompression therapy to other patients?	A B C D E
13.	Can you briefly describe what you think are advantages of cryocompression therapy?	Open text
14.	Can you briefly describe what you think are disadvantages of cryocompression therapy?	Open text
15.	From 0 to 10 how would you rate cryocompression treatments you received? (Mean ± SD)	0 – 10

Number of respondents = 35. Response categories: 1 = strongly agree; 2 = mildly agree; 3 = neutral; 4 = mildly disagree; 5 = strongly disagree.
IQR: Interquartile range; SD: standard deviation.

Table 6. Nursing staff questionnaire.

	Question	Answer options
1.	Was the GRS hip/groin wrap technically easy to apply?	A B C D E
2.	Was the GRS hip/groin wrap easy to apply to postoperative hip fracture patients?	A B C D E
3.	Did you apply the GRS hip/groin wrap alone?	Yes / No
4.	Was the control unit easy to operate?	A B C D E
5.	If the GRS works do you think that the application 4 times 30 minutes a day is feasible?	A B C D E
6.	Were you able to administer all the treatments that were required?	A B C D E
7.	Would you recommend the GRS to patients?	A B C D E
8.	Do you think patients recovered faster because of the GRS	A B C D E
9.	Should the GRS be apart of standard hip fracture treatment?	A B C D E
10.	Can you briefly describe what you think are advantages of cryocompression therapy?	Open text
11.	Can you briefly describe what you think are disadvantages of cryocompression therapy?	Open text

Number of respondents = 51. Response categories: 1 = strongly agree; 2 = mildly agree; 3 = neutral; 4 = mildly disagree; 5 = strongly disagree.
IQR: Interquartile range; SD: standard deviation. GRS: Game Ready System.

Statistics

Analysis of outcome parameters

All statistical analyses will be computed using the SPSS statistical package (IMB SPSS, Inc., Release 20.0.0.0, 64-bit edition). Statistical analysis will be performed according to the intention-to-treat principle. Baseline characteristics will be described in accordance with CONSORT guidelines[41] using means and standard deviation in case of normal distribution, and medians and interquartile ranges otherwise. Continuous variables will be checked for normality, visually and by using the "Shapiro Wilk" test. Our primary analysis focuses on the differences in NRS-pain scores between the study groups 24 hours postoperative and will be analyzed by use of Student's T-tests or Mann-Whitney U tests in case of skewed distribution. To assess treatment effect over time, a mixed model analysis for repeated measures will be performed. This model allows missing data and adjustment for serious confounders and interactions. In case of skewed distributions and outliers non-parametric variants will be used (e.g. Mann Whitney U tests, Friedman tests). Secondary continuous outcome measures (EQ-5D-3L, SF-12, DRS-R-98 and hemoglobin) will be compared by use of Student's t-tests or Mann Whitney U tests. Ordinal variables will be analyzed using the Mann-Whitney test. Chi-squared tests will be performed in case of categorical variables. In case of important confounding, analysis will be adjusted to correct for these factors (by use of multiple regression analysis, linear or logistic). A p-value of < 0.05 will be considered statistically significant.

Sample size calculation

Sample size calculation is based on the primary outcome measure, NRS-pain at 24 hours postoperatively. Subsequently, a mean NRS-pain score for postoperative day one was drawn from the 'Spaarne Hospital' electronic patient dossier digital databanks and a standard deviation of 2.2 was calculated for pooled internal fixation and (hemi) arthroplasty for cervical and peritrochanteric fractures. The minimal clinical relevant difference for NRS-pain is 1.3 out of 10[53]. The alpha was set at 5% with a power of 90% due to anticipated heterogeneity in regard to operating techniques, general care protocols but predominantly in regard to pain protocols between the participating centers (Table 2). An expected NRS failure rate of 10% is anticipated together with expected missing data a drop out of 22.3% is computed. The total number of included patients will be 160.

Discussion

This multicenter study will evaluate the efficacy of intermittent application of continuous-flow cryocompression therapy administered in the first 72 postoperative hours after hip fracture surgery. Due to the multicenter design of the study the results can easily be translated to the general care of hip fracture patients. Conversely, some inequality in local hospital pain protocols is present but conversion through accepted algorithms[59, 60] make comparison feasible. Spinal anesthetized patients could have advantages over the general anesthesia group in the acute hours post-surgery in regard to pain scores and analgesic use since the effect of the latter ends immediately after surgery in contrast to the former that takes a few hours to wear off completely.

The varying types of fractures have different characteristics and demand a different surgical approach. Peritrochanteric fractures quite often have more extensive bony trauma and less soft-tissue trauma, and after surgical stabilization with a DHS or IMHN fracture micro motion leads to prolonged and greater dynamic pain[6]. Conversely, in (hemi) arthroplasty the fracture-site is removed completely at the expense of increased (surgical-induced) soft-tissue trauma. Hence the efficacy of CFC therapy is likely to vary in patients with varying extent of soft-tissue trauma. Therefore stratification is implemented according to type of surgery that divides patients with greater surgical trauma ((hemi) arthroplasty) and lesser surgical trauma (cannulated screws, DHS, IMHN).

By nature, delirium is known to fluctuate over the day and a single DRS-R-98 measurement might not reach sufficient sensitivity for diagnosis. However with longitudinal follow-up of three times daily DOS-scores this problem is overcome for the most part. Delirium severity is assessed with more information gathered from family and personnel, over the past 24 hours thereby making assessment adequate.

Currently four of the five participating hospitals have a geriatric trauma unit, in one of the hospitals with a geriatric trauma unit the care of the surgical geriatric patient is transmitted to the geriatrician in full except for wound assessments and related care. At this ward all patients receive prophylactic haloperidol 1 mg daily, and more if needed. At the hospital without a geriatric trauma unit a geriatrician is not readily available and unable to attend the clinic on a daily basis. Confounding of secondary outcomes as delirium, psychotropic medication and functional outcome can therefore not be ruled out.

When compared to the preoperative values, hemoglobin levels in collected blood samples at postoperative day one are usually much lower than one would expect from the observed blood loss during surgery. This gap called ‘the hidden blood loss’ is thought to arise from,

amongst others gastro-intestinal bleeding[22, 79]. The amount of (hidden) blood loss is more severe when oral anticoagulants or thrombocyte aggregation inhibitors compromise a patient's hemostasis and this loss may continue throughout the early postoperative period. Hemoglobin measured at postoperative day one is most likely, as opposed to the preoperative values, significantly diluted, thereby underestimating the actual hemoglobin concentration. Combined with the possibility of hidden blood loss throughout the hospital stay, care has to be taken when comparing the value of postoperative day one to day three.

The functional outcome assessment of patients who are not allowed to bear weight at the time of discharge is postponed to the outpatient clinic. At this visit they are allowed to bear weight for the first time in weeks. Testing directly after weight bearing for the first time can introduce confounding.

Finally, care has to be taken to interpret the allocation to rehabilitation facilities. For instance patients who have sufficient family members who can help out at home are more likely to be discharged home with or without homecare rather than be assigned to a rehabilitation clinic or nursing home with rehabilitation facilities.

Conclusion

The present study will provide evidence for the efficacy of continuous-flow cryocompression therapy applied after hip fracture surgery. Due to the duplicate trauma this condition encompasses these patients, who are generally aged 70 years and over, are expected to benefit most. Furthermore treatment feasibility is assessed and consequently, recommendations are made about which settings are best to employ.

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Chapter 5

Postoperative continuous-flow cryocompression therapy in the acute recovery phase of hip fracture surgery: a randomized controlled clinical trial

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Abstract

Background: The acute recovery phase after hip fracture surgery is often complicated by severe pain, postoperative blood loss with subsequent transfusion, and delirium. Prevalent comorbidity in hip fracture patients limit the use of opioid-based analgesic therapies, yielding a high risk for inferior pain treatment. Postoperative cryotherapy is suggested to provide an analgesic effect, and to reduce postoperative blood loss. In this prospective, open-label, parallel, multicentre, randomized controlled, clinical trial, we aimed to determine the efficacy of continuous-flow cryocompression therapy (CFCT) in the acute recovery phase after hip fracture surgery.

Methods: Patients with an intra or extracapsular hip fracture scheduled for surgery were included. Subjects were allocated to receive postoperative CFCT or usual care. The primary endpoint was numeric rating scale (NRS) pain the first 72 postoperative hours. Secondly, analgesic use; postoperative haemoglobin change and transfusion incidence; functional outcome; length of stay; delirium incidence; location of rehabilitation; patient-reported health outcome; complications and feasibility were assessed.

Results: Sixty-one subjects in the control group, and 64 subjects in the CFCT group were analysed. Within the CFCT group, post treatment NRS pain declined 0.31 (p=0.07) at 24 h, 0.28 (p=0.07) at 48 h, and 0.47 (p=0.002) at 72 h relative to pre treatment NRS pain. Sensitivity analysis at 72 h showed that NRS pain was 0.92 lower in the CFCT group when compared to the control group (1.50 vs. 2.42; p=0.03). Postoperative analgesic use was comparable between groups. Between postoperative day 1 and 3 haemoglobin declined 0.29 mmol/l in the CFCT group and 0.51 mmol/l in controls (p=0.06), and transfusion incidence was comparable. The timed up and go test and length of stay were also comparable between both groups. Complications, amongst delirium and cryotherapy-related adverse events were not statistically significantly different. Discharge locations did not differ between groups. At outpatient follow-up subjects did not differ in patient-reported health outcome scores. Subjects rated CFCT satisfaction with an average of 7.1 out of 10 points.

Conclusions: No evidence was recorded to suggest that CFCT has an added value in the acute recovery phase after hip fracture surgery. If patients complete the CFCT treatment schedule, a mild analgesic effect is observed at 72 h.

Introduction

Hip fractures frequently occur in the elder population, and global incidence is steadily rising[1]. Mainly due to aging of the population, global incidence figures are projected to increase by 1-3% annually the coming decades[1]. Early surgical fixation with direct postoperative mobilization remains the cornerstone of hip fracture treatment in nearly all cases, and targets preservation of pre-fracture functional status.

The severe postoperative pain hip fracture patients experience has led to various treatment options ranging from nerve blockades, loco-regional opioid administration, and to systemic (opioid) analgesia therapy[2,3]. The multitude of treatment options illustrates that adequate pain control continues to be a challenge in this condition. Most elderly patients experience physiological decline in organ function (such as renal or hepatic function) that can alter the pharmacology of analgesics[4]. In addition, comorbidities and polypharmacy render elderly susceptible to drug interactions and adverse events, which puts them at risk for inferior pain treatment[4,5]. The need for adequate pain relief is evident, as inferior pain treatment has been associated with delayed ambulation, which leads to an extended hospital admission, to poorer short and mid-term functional outcome and development of postoperative delirium[6,7]. Taken together, these altered pharmacodynamics and kinetics narrow the therapeutic window, and combined with the painful nature of a hip fracture this leads to increased difficulty in providing adequate analgesia to hip fracture patients[2].

The injury sustained during a hip fracture causes fracture site bleeding that render 40% of admitted hip fracture patients anaemic[8]. The additional blood loss that fracture repair encompasses increases this figure to 93%[8]. This high incidence of anaemia is problematic since it negatively impacts length of stay (LOS), readmission rates, and odds of death[8]. However, erythrocyte transfusion delays wound healing and increases risk of infection, hence the need for transfusion should be carefully considered, or avoided completely if feasible[9]. Continuous-flow cryocompression therapy (CFCT) combines the flow of ice-cold water with a dynamic intermittent compression adjunct aiming to result in haemostasis as well as providing analgesia in a non-pharmacological manner. It is mostly applied after musculoskeletal soft tissue trauma or after semi-elective musculoskeletal surgery such as arthroplasty, with ambiguous results. In two studies with total knee arthroplasty (TKA) patients, CFCT reduces postoperative pain and postoperative blood loss, while others found no apparent effect[10–13]. In total hip arthroplasty (THA) patients CFCT reduces morphine consumption and mitigates postoperative haemoglobin decline, while others demonstrated a shortening of admission time[14–16]. A hip fracture is generally accompanied by soft tissue

trauma that is aggravated by subsequent surgical fixation[17]. As hip fracture patients have duplicate trauma (the fracture and the surgical fixation), experience severe pain, and have fracture site bleeding with related inflammation, these patients are expected to benefit most from CFCT.

To date no studies have researched the efficacy of CFCT in the acute postoperative recovery phase of hip fracture surgery. We hypothesized that: 1) CFCT will lower perceived pain levels and analgesic use; and 2) will reduce postoperative blood loss and transfusion incidence; and 3) reduced pain by CFCT will lead to lower delirium incidence, and 4) will enhance functional recovery, leading to shorter LOS. We aimed to determine the analgesic efficacy of CFCT in the acute postoperative recovery phase of hip fracture surgery. Secondly, we aimed to determine the effect on postoperative blood loss, short-term functional outcome parameters, and assess feasibility.

Methods

The Medical Ethical Committee 'METC Noord-Holland', Alkmaar, The Netherlands (date: September 29, 2014; reference no: M014-013) approved the study and after written informed consent was obtained, 126 subjects were enrolled in this open-label, parallel, multicentre, randomized controlled, clinical superiority study within 14 months time (December 2014 and January 2016) at eight orthopaedic surgery, general surgery and/or geriatric departments in four hospitals. The study was registered at trialregister.nl with identifier NTR4152.

The study protocol has been published in full detail[18]. In brief, subjects with an intra or extracapsular hip fracture who met the criteria were recruited at the accident and emergency department and randomised post surgery (Table 1). Intramedullary hip nail (IMHN), dynamic hip screw (DHS) and cannulated hip screws (CHS) were grouped for block randomization, as were total hip arthroplasty (THA) and hemiarthroplasty (HA). Subjects were allocated to receive CFCT during the first 72 hours (h) postoperative or to receive usual care without CFCT. During this timeframe subjects allocated to the intervention group received 10 to 12 CFCT treatments of 30 minutes each, and were asked to value pre and post treatment pain at rest by using the numeric rating scale (NRS; range 0-10). Subjects allocated to the control group were only asked to value NRS pain at rest. The nurse applied the device that administered the CFCT ('Game Ready system' (GRS); Coolsystems Alameda, California) using the hip/groin wrap. Through this anatomically designed hip/groin wrap covering most of the thigh and pelvis up to the iliac crest, the GRS simultaneously delivers both adjustable continuous-flow cryotherapy and intermittent compression through a portable control unit

filled with ice and water. The machine has four pressure settings: no pressure, low pressure (5-15 mm Hg), medium pressure (5-50 mm Hg), and high pressure (5-75 mm Hg). Temperature can be adjusted between 4 and 13 °C and is indicated by one, two or three snowflakes. If feasible, the lowest temperature was started and maintained throughout the study, and pressure was started at *'low'* and was increased stepwise with one pressure increment per 4 treatments administrated. Haemoglobin values are obtained from blood samples at postoperative day (POD) one and three. Blood loss was calculated by subtracting haemoglobin concentration from POD three and POD one. Specifically designed feasibility questionnaires were completed by CFCT subjects at discharge to document experiences with CFCT[18].

Table 1. Subject criteria.

Inclusion criteria	Exclusion criteria
* Intra or extracapsular femur fracture	* Fractures at multiple foci
* Age ≥18 years	* Open fracture/wounds[a]
* Informed consent or proxy consent	* Acetabular fracture
	* (Suspicion of) concomitant malignancy
	* BMI > 40
	* Unwilling to give proxy consent
	* Preoperative osteosynthesis materials in situ in the ipsilateral leg above knee level
	* Morphine allergy or dependence
	* ASA ≥ 4
	* Cryoglobulinemia
	* M. Raynaud
	* Central neuromuscular disorder
	* Absent ADP/ATP pulsations in the injured extremity
	* Active deep vein thrombosis
	* Suspected pulmonary embolism
	* Patient delay > 24 hours
	* NYHA-class ≥ 3
	* IQ-CODE score ≥ 4.6[b]
	* Long-acting femoral blocks
	* Use of local infiltration anaesthesia
	* Postoperative haemodynamic instability

[a]Open wounds unable to close per primam. [b]The IQ-CODE is only administered if the clinician has doubts about the cognitive status of the subject. BMI: body mass index; ASA: American Society of Anesthesiologists; ADP: dorsal pedal artery; ATP: posterior tibial artery; NYHA: New York Heart Association; IQ-CODE: Informant Questionnaire for the Cognitive Decline in the Elderly.

Protocol deviations

Deviations occurred from the prespecified protocol[18]. The long acting, non-protocol approved, oxycontin opioid was frequently prescribed. Physicians that were not actively involved in the study and who supervised daily care for study subjects were poorly informed about newly enrolled subjects, and the restrictions that were associated with participation. The high incidence of non-protocol approved analgesics precluded the use of an equianalgesic dosage conversion to facilitate comparison of the analgesic outcome parameter. Instead we reported incidence of long-acting analgesics (oxycontin), and incidence of short-acting analgesics (diclofenac, oxycodone, morphine) separately.

Delirium rating scale revised 1998 (DRS-R-98) scores were inconsistently registered in a way that precluded meaningful analysis[19]. Personnel assigned to administer the DRS-R-98 were often not available due to busy daily routine. Instead the delirium observation screening (DOS) score that was registered three times daily was used[20]. A delirium was redefined as a DOS ≥ 3 or a delirium established by a geriatrician regardless of the DOS score. Initially the LOS outcome was defined as: NRS-pain score below 5 without need for parenteral analgesics and an Elderly Mobility Scale score 14 out of 20 or higher[21]. Due to inconsistent daily registration of the Elderly Mobility Scale the LOS outcome is redefined as: the subject discharge time subtracted from the time the index surgery ended. Due to missing data the following other parameters were omitted from analysis: cumulated NRS pain analysis, mini mental state examination, de Morton mobility index, short physical performance battery, and timed up and go test (TUGT) at outpatient visit.

In order to determine if cryotherapy could have deleterious effects on early stages of callus formation, all radiographs of subjects treated with CHS, a DHS or an IMHN were reviewed to determine the presence of callus.

Statistics

Within the primary power analysis that was undertaken during the preparation of the study protocol an α 0.05, a β of 0.90 and a drop out of 22% was used, from which a sample size of 160 subjects was calculated[18]. The study was relatively overpowered because it was expected that due to the multicentre design a part of the data could not be used for analysis. After consultation with two independent methodologists, data from subjects that had missing or incomplete informed consent forms (and were unobtainable from subjects) were omitted from data analysis and this manuscript. A regular power analysis with an α 0.05, a β of 0.80 and a

10% drop out would have consisted of 101 subjects, hence in its current form the study is adequately powered.

All variables were summarized with standard descriptive statistics including mean and standard deviation (SD) or medians with interquartile ranges (IQR), and frequencies. Univariate analysis was performed to compare baseline characteristics.

Due to an inequality in drop out rate between the control and the intervention group, and a resulting imbalance in missing data, the missing data was imputed by the use of the last observation carried forward (LOCF) method[22]. Primary efficacy analysis was performed in the intention to treat population according to the LOCF protocol. Sensitivity analyses were performed by use of per protocol analyses without imputation. Statistical analysis of NRS pain was performed by use of multivariate linear regression analysis at three endpoints, 24 h, 48 h and 72 h postoperatively. Unadjusted as well as adjusted coefficients were calculated to assess a treatment effect. NRS pain registered 5 h before and 5 h after the time points 24 h, 48 h and 72 h were grouped for analysis. Additional mixed-model repeated-measures analysis of covariance was performed to assess treatment effect during the first 24 h and 72 h. Potential confounders for the analyses were gender, age, body-mass index (BMI), surgery type, preoperative carbasalate calcium use, anaesthesia type, and centre. Haemoglobin change between first and third postoperative day was analysed by use of multivariate regression analysis, and adjusted for potential confounders (gender, age, BMI, preoperative carbasalate calcium use, wound drain, and static compressive dressing use). Other secondary continuous outcome measures EuroQol-5D-3L (EQ-5D-3L) and Short Form 12 (SF-12) were compared by use of Student's t-tests or Mann Whitney U tests. Ordinal variables were analysed using the Mann-Whitney test. Chi-squared tests were performed in case of categorical variables. A p-value of < 0.05 was be considered statistically significant.

Results

Sixty-one subjects in the control group and 64 subjects in the CFCT group received the allocated treatment, and were analysed (Figure 1). Both groups had similar demographic characteristics (Table 2).

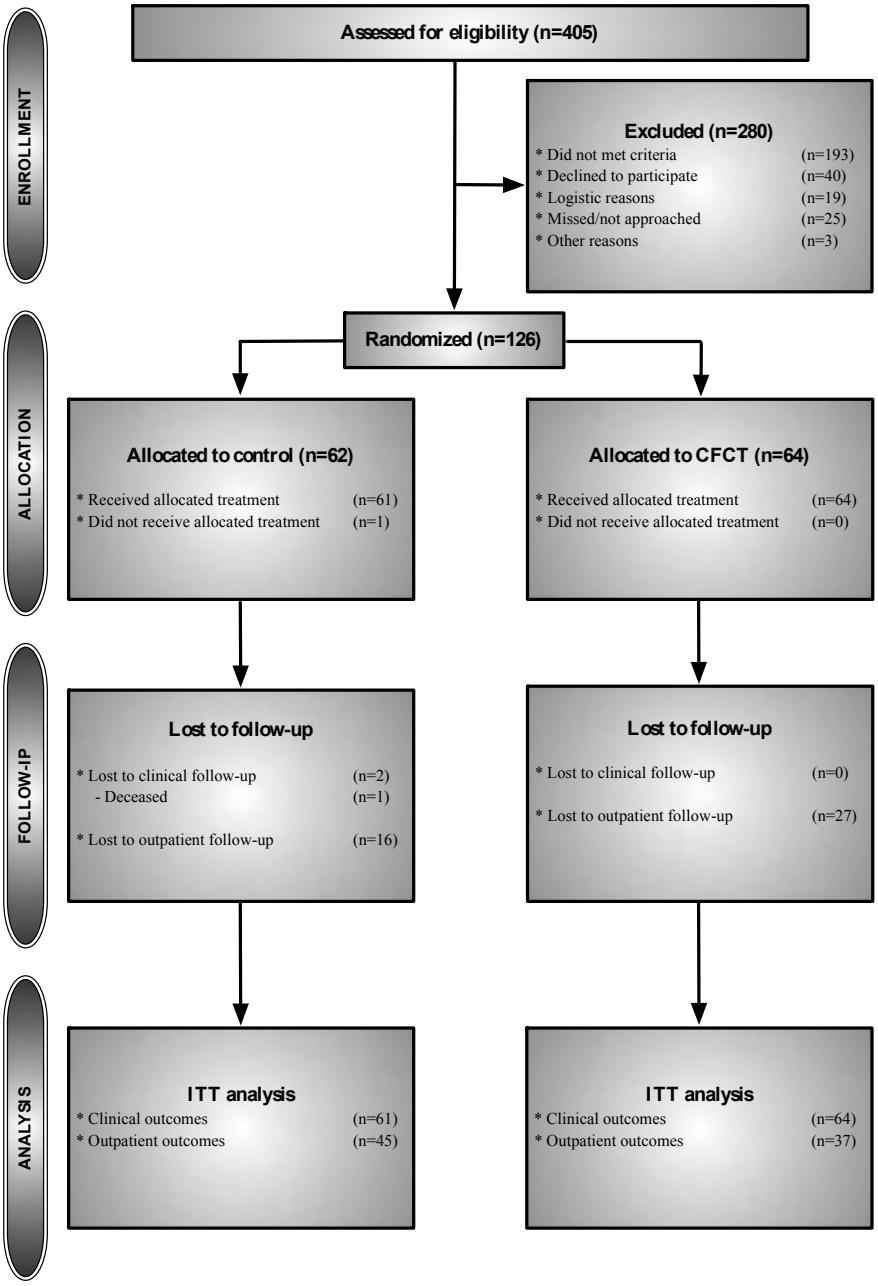
Table 2. Subject characteristics.

Variable	**Control** (n=61)	**CFCT** (n=64)	***p*-value**
Male, *n* (%)	22 (36%)	15 (23%)	0.12
Age, years	77.2 (10.1)	80.0 (10.9)	0.13
BMI, kg/m^2	24.6 (4.1)	24.4 (4.1)	0.74
NMS pre-fracture, median (IQR)	9 (4-9)	9 (3-9)	0.06
ASA class, *n* (%)			
- ASA 1	6 (10%)	10 (16%)	0.60
- ASA 2	35 (57%)	33 (52%)	
- ASA 3	20 (33%)	21 (33%)	
Intracapsular fracture, *n* (%)	35 (57%)	34 (53%)	0.63
Spinal anaesthesia, *n* (%)	42 (69%)	47 (73%)	0.57
Type of surgery, *n* (%)			
- DHS/CHS	16 (26%)	16 (25%)	0.99
- HA/THA	19 (31%)	20 (31%)	
- IMHN	26 (43%)	28 (44%)	
Carbasalate calcium use, *n* (%)	17 (28%)	13 (20%)	0.32
Pain at A&E department, NRS	5.04 (1.9)	5.02 (2.0)	0.97

Data are presented as mean and standard deviation unless stated otherwise. CFCT: continuous-flow cryocompression therapy; SD: standard deviation; BMI: body mass index; NMS: new mobility score; IQR: interquartile range; ASA: American Society of Anesthesiologists; DHS: dynamic hip screw; CHS: cannulated hip screws; HA: hemiarthroplasty; THA: total hip arthroplasty; IMHN: intramedullary hip nail; NRS: numeric rating scale.

The CFCT group received an average of 9.1 treatments during the first 72 h, and in the control group an average of 9.6 NRS pain scores was registered within the same period. NRS pain was comparable between groups at 24 h, 48 h and 72 h when analysed by the LOCF method (Table 3). The sensitivity analyses at 24 h and 48 h showed comparable NRS pain between groups. Sensitivity analysis at 72 h showed that post treatment NRS pain in the CFCT group was significantly lower than the NRS pain in the control group (2.42, SD 1.9, n=48 compared to 1.50, SD 1.8, n=34; p=0.03). Mean pre and post treatment NRS pain during the first 24 h and 72 h was also comparable between groups (Table 3). In the CFCT group the mean decline in NRS pain before and after treatment was 0.31 (95%CI: -0.02; 0.65; p=0.07) at 24 h, 0.28 (95%CI: -0.02; 0.58; p=0.07) at 48 h, and 0.47 (95%CI: 0.18; 0.76; p=0.002) at 72 h. Incidence of short and long acting analgesic use was comparable between groups (Table 4).

Figure 1. GRAPES trial flowchart.



CFCT: continuous-flow cryocompression therapy; ITT: intention-to-treat analysis.

Table 3. The effect of continuous flow cryocompression therapy on numeric rating scale pain.

Variable	Univariate (t-test)				
	Control (n=61)	**CFCT** (n=64)	***p*-value**	**Adjusted ß-coefficient**[b]	***p*-value**
NRS pain[a]					
- At 24 h pre treatment	2.61 (1.94)	2.70 (1.86)	0.78	-0.01 (-0.75; 0.73)	0.99
- At 24 h post treatment	2.61 (1.94)	2.39 (1.94)	0.54	-0.23 (-0.98; 0.52)	0.55
- At 48 h pre treatment	1.92 (1.82)	2.27 (2.07)	0.32	0.26 (-0.49; 1.01)	0.50
- At 48 h post treatment	1.92 (1.82)	1.98 (1.90)	0.84	0.06 (-0.66; 0.78)	0.87
- At 72 h pre treatment	2.15 (1.84)	2.34 (1.89)	0.56	0.07 (-0.62; 0.76)	0.84
- At 72 h post treatment	2.15 (1.84)	1.88 (1.94)	0.42	-0.42 (-1.13; 0.28)	0.23
Mean NRS pain[a]					
- During 24 h pre treatment	2.62 (2.26)	2.99 (2.05)	0.37	-0.12 (-0.64; 0.41)	0.66
- During 24 h post treatment	2.62 (2.26)	2.61 (2.12)	0.75	0.15 (-0.38; 0.68)	0.58
- During 72 h pre treatment	2.31 (1.99)	2.56 (2.02)	0.27	-0.06 (-0.73; 0.61)	0.85
- During 72 h post treatment	2.31 (1.99)	2.22 (2.04)	0.81	0.21 (-0.49; 0.90)	0.55

Data are presented as mean and standard deviations unless stated otherwise. [a]Analysed with last observation carried forward method. [b]Adjusted for: gender, age, BMI, carbasalate calcium use, type of surgery, type of anaesthesia, and centre. CFCT: continuous-flow cryocompression therapy; NRS: numeric rating scale; h: hours.

Erythrocyte transfusion incidence administered during enrolment was comparable between groups (Table 4). Subjects that received an erythrocyte transfusion were omitted from haemoglobin decline outcome analysis. Haemoglobin decline between POD 1 and POD 3 was 0.29 mmol/l in the CFCT group and 0.51 mmol/l in the control group (p=0.06; Table 4). The time until active wound discharge had ceased was comparable between groups. One control subject was discharged with active wound discharge, which had ceased at the outpatient follow-up visit (Table 4). Delirium incidence and haloperidol use was comparable between groups (Table 4). The TUGT time was comparable between groups (Table 4). The TUGT was completed in 50 subjects, of the remainder 7 were not allowed to bear weight, in 22 subjects the test was not administered due to unclear reasons, 40 subjects were unable, and another 6 subjects refused to perform the test during admission. No difference in LOS was observed between groups (Table 4). Discharge location was comparable between the control and CFCT group, 23 control subjects compared to 19 CFCT subjects were discharged homebound, and 35 control subjects compared to 44 CFCT subjects were discharged to a nursing facility. Three subjects were transferred to another hospital due to geographical preference, and one discharge location was unknown.

The outpatient study follow-up visit was completed in 83 subjects (Figure 1), and consisted of 38 physical visits (58% control subjects and 42% CFCT subjects), and 45 follow-ups via telephone (51% control subjects and 49% CFCT subjects). Fifteen subjects (6 controls, 9

CFCT subjects) declined the outpatient study follow-up visit; thirty follow-up visits were missed by the investigator (12 controls, 18 CFCT subjects). In the 6 weeks follow-up interval no subject deceased. NRS pain and analgesic use incidence were comparable at the outpatient study visit (Table 4). The patient reported outcome measures SF-12 and EQ5D-3L were comparable between groups (Table 4). In the subjects that received CHS, a DHS or an IMHN visible callus incidence on radiographs was comparable at the outpatient follow-up visit (Table 4). In 30 subjects no radiographs were acquired at the outpatient follow-up visit, and in 25 subjects an (hemi) arthroplasty was performed.

Thirty-five (55%) CFCT subjects completed a feasibility questionnaire (Table 5). Subjects stated it felt as they recovered faster by CFCT, and preferred CFCT to analgesics but would prefer not to have been treated longer or more frequent than the current treatment schedule. Subjects graded CFCT with an average 7.1 points (scale 0-10), and would recommend CFCT to other patients. Eighteen (28%) CFCT subjects reported discomfort. Ten (15.6%) CFCT subjects dropped out prematurely due to discomfort of the cold adjunct of which two subjects had developed blisters, and one subject contracted a pressure ulcer from the CFC hose. Blister development was also observed in two control subjects. The blisters in the four subjects were located near applied bandages, however these subjects had no known allergy for bandages. No statistical differences were observed in complication rate between groups (Table 4).

Nursing staff found the GRS hip/groin wrap technically easy to apply, and to control unit easy to operate (Table 6). They required an average of 12.8 minutes to prepare the control unit, apply and remove the wrap for a single treatment. Due to daily routine 35% of nursing staff found the current treatment schedule not to be feasible in daily practice, and would recommend reducing the frequency. Another 35% reported that the application/removal of the wrap was painful for subjects.

Table 4. The effect of continuous flow cryocompression therapy on secondary outcome parameters.

Variable	*n*	Control	*n*	CFCT	*p*-value
Incidence of analgesic use, *n* (%)					
Short acting[a]					
- 0 – 24 h	60	41 (68%)	63	38 (60%)	0.35
- 24 – 48 h	60	26 (43%)	63	20 (32%)	0.18
- 48 – 72 h	60	26 (43%)	63	18 (27%)	0.09
Long acting[b]					
- 0 – 24 h	60	14 (23%)	63	12 (19%)	0.56
- 24 – 48 h	60	14 (23%)	63	8 (13%)	0.12
- 48 – 72 h	60	15 (25%)	63	8 (13%)	0.08
Incidence of haloperidol use, *n* (%)					
- 0 – 24 h	60	8 (13%)	63	15 (24%)	0.10
- 24 – 48 h	60	7 (12%)	63	16 (25%)	0.04
- 48 – 72 h	60	7 (12%)	61	11 (18%)	0.23
Erythrocyte transfusion incidence, *n* (%)	61	9 (15%)	64	9 (14%)	0.91
Haemoglobin decline[c], mmol/l	28	0.51 (0.53)	40	0.29 (0.42)	0.06
Active wound discharge, days	60	0.92 (1.43)	64	0.75 (1.68)	0.55
Delirium incidence, *n* %	61	14 (23%)	64	13 (20%)	0.72
Timed up and Go test, seconds	25	57.2 (46.4)	25	56.5 (44.3)	0.96
Length of stay, days	61	6.0 (4.5)	64	6.2 (5.3)	0.88
SF-12 physical subdomain, points	31	41.4 (6.5)	26	41.2 (6.5)	0.89
SF-12 mental subdomain, points	31	42.0 (5.8)	26	41.9 (5.8)	0.97
EQ-5D, points	40	0.71 (0.19)	36	0.72 (0.22)	0.70
Callus formation on radiograph[d], *n* (%)	35	32 (91%)	34	33 (97%)	0.32
Pain at outpatient visit, NRS	39	1.08 (1.8)	36	1.42 (2.2)	0.47
Analgesic use at outpatient visit, *n* (%)	43	17 (40%)	36	15 (42%)	0.85
Complications, *n* (%)					
- Urinary tract infection	61	10 (16%)	64	11 (17%)	0.91
- Pneumonia	61	3 (5%)	64	5 (8%)	0.51
- Wound infection	61	0 (0%)	64	3 (5%)	0.09
- Delirium	61	7 (12%)	64	8 (13%)	0.86
- Non-infectious systemic	61	11 (18%)	64	11 (17%)	0.54

Data are presented as mean and standard deviations unless stated otherwise. [a]Short acting: diclofenac, short acting opioids such as oxycodone, parental morphine (amongst is patient controlled analgesia). [b]Long acting: oxycontin. [c]Decline between postoperative day 1 and day 3; Corrected for gender, age, BMI, preoperative carbasalate calcium use, wound drain, and static compressive dressing use. [d]Subjects treated with dynamic hip screw, cannulated hip screws, or intramedullary hip nail only. CFCT: continuous-flow cryocompression therapy; SF: Short Form; EQ: EuroQol; NRS: numeric rating scale.

Table 5. Subject satisfaction questionnaire about continuous-flow cryocompression therapy experiences.

	Question	Median; IQR
1.	Did you feel that the pain reduced when treated with cryocompression therapy?	2.0; 2
2.	Would you rather have cryocompression treatment than analgesic pain treatment?	1.0; 2
3.	The standard setting used was the coldest; did you like this temperature setting?	3.0; 2
4.	Did you request the temperature setting to be upped?	Yes 15.4%
5.	Did you like the dynamic pressure adjunct?	2.0; 2
6.	The pressure adjunct was elevated every 4 treatments, was the pace to fast?	4.0; 3
7.	After treatment the muscles are cooled, did this hinder you in moving around outside of bed?	4.0; 3
8.	Would you have liked to be treated more often per day than 4 times?	4.0; 2
9.	Would you have liked to be treated longer than 30 minutes per cycle?	5.0; 2
10.	Would you have liked to be treated longer than the first 72 hours postoperative?	5.0; 2
11.	Did you feel like you recovered faster with cryocompression therapy?	3.0; 2
12.	Would you recommend the use of cryocompression therapy to other patients?	1.0; 2
13.	Can you briefly describe what you think are advantages of cryocompression therapy?	Open text
14.	Can you briefly describe what you think are disadvantages of cryocompression therapy?	Open text
15.	From 0 to 10 how would you rate cryocompression treatments you received? (Mean ± SD)	7.1 ± 1.73

Number of respondents = 35. Response categories: 1 = strongly agree; 2 = mildly agree; 3 = neutral; 4 = mildly disagree; 5 = strongly disagree. IQR: Interquartile range; SD: standard deviation.

Table 6. Nurse staff questionnaire about continuous-flow cryocompression therapy experiences.

	Question	Median; IQR
1.	Was the GRS hip/groin wrap technically easy to apply?	2.0; 0
2.	Was the GRS hip/groin wrap easy to apply to postoperative hip fracture patients?	3,0; 2
3.	Did you apply the GRS hip/groin wrap alone?	2.0; 2
4.	Was the control unit easy to operate?	2.0; 0
5.	If the GRS works do you think that the application 4 times 30 minutes a day is feasible?	4.0; 2
6.	Were you able to administer all the treatments that were required?	2.0; 1
7.	Would you recommend the GRS to patients?	3.0; 1
8.	Do you think patients recovered faster because of the GRS?	3.0; 0
9.	Should the GRS be apart of standard hip fracture treatment?	3.0; 0
10.	Can you briefly describe what you think are advantages of cryocompression therapy?	Open text
11.	Can you briefly describe what you think are disadvantages of cryocompression therapy?	Open text

Number of respondents = 51. Response categories: 1 = strongly agree; 2 = mildly agree; 3 = neutral; 4 = mildly disagree; 5 = strongly disagree. IQR: Interquartile range; SD: standard deviation. GRS: Game Ready System.

Discussion

No clinical difference in analgesic use, postoperative haemoglobin change, transfusion incidence, functional outcome, LOS, delirium incidence, haloperidol use, location of rehabilitation, and patient-reported health outcome was found between the CFCT and control group. At 72 h, pain, as measured by NRS, was significantly less suggesting that CFCT may have an advantage on pain at 72 h postoperative, but has no added value in the direct postoperative recovery phase of hip fracture. Since subjects valued CFCT, and no difference in complication rate was observed it could be considered safe to apply to hip fracture patients.

Continuous-flow cryocompression therapy reduced post treatment pain scores significantly at 72 h postoperative. Because of the study design, no information on NRS after 72 h was collected. This is the first randomized clinical trial reporting on the efficacy of cryotherapy in postoperative hip fracture patients. Cryotherapy is well researched in THA for end-stage osteoarthritis. Three randomized controlled trials (RCT's) and one prospective trial assessed the analgesic efficacy of cryotherapy[11,14–16]. Two found cryotherapy to reduce pain and analgesic use, while others demonstrate continuous applied cryotherapy to have no analgesic effect[11,14–16]. Although in our THA study CFCT reduces postoperative haemoglobin decline, we were unable to demonstrate a statistical significant difference in the current study[14]. Others measured drain output and haemoglobin during the first 48 h, and found it not to be affected by cryotherapy[11]. In summary, two studies found a mild analgesic effect of cryotherapy while the other two reports did not demonstrate any difference in pain. Thus, in a limited number of studies diverging data exists about the effect of cryotherapy in THA patients.

The effect of cryotherapy is more thoroughly investigated after TKA. A meta-analysis that included 11 RCT's with 793 TKA's states that application of cryotherapy results in small benefits in blood loss and short term knee range of motion, but does not reduce pain, analgesic demands, swelling or LOS[23]. A more recent meta-analysis that compared CFCT to cryotherapy alone in 552 patients (10 RCT's) states that CFCT has beneficial effects on pain perception and postoperative knee swelling during the first three days after surgery[24]. The effect of cryotherapy after TKA is moderate, and the compression adjunct seems to somewhat enhance cryotherapy' efficacy, but the same cannot be concluded from the sparse data in THA patients.

A major difference between the knee and hip joint is the presence of subcutaneous fat (and muscle) tissue. While the knee virtually lacks a fat layer, a layer of up to several centimetres thick surrounds most hip joints. In our multivariate regression analysis BMI was not a significant confounder. However in healthy individuals, subcutaneous adipose tissue

thickness strongly delays intramuscular cooling time at the anterior thigh[25]. Subjects with a skin-fold of less than 20 mm require a 25 minute crushed ice bag treatment to reduce intramuscular temperature to 7°C at 1 cm below the fat layer, while it takes subjects with a skin fold of 31-40 mm as long as 60 minutes to reach the same temperature[25]. The current cryotherapy machine lowers the temperature to 23°C at 1.5 cm below the subcutaneous fat layer at high pressure in healthy individuals with average skin folds of 11.3 mm[26]. Given the reported inverse relationship between thigh skinfold thickness and tissue temperature decline by cryotherapy, cryotherapy does not seem to be efficacious in cooling the injured muscles in obese hip fracture patients.

Two theories are proposed on how cryotherapy applies the physiological effects of cooling to injured tissue. The first theory states cryotherapy to exert a direct analgesic effect via a decrease in nerve conduction velocity that consequently reduces pain perception[27]. Ice packs increase pain threshold and pain tolerance with a simultaneous decrease in nerve conduction velocity of 33% at 10°C skin temperature in the tibial nerve of the ankles of healthy subjects[27]. Secondly, cryotherapy is suggested to alleviate pain indirectly by reducing inflammation via an inhibition of tissue metabolism. Although no data is available on cryotherapy-induced decline in metabolism in muscle cells, it is known that synovial fluid lactate concentrations remain stable despite a decrease in blood flow when cryotherapy is applied in patients recovering from knee arthroscopy, suggesting a decrease in energy requirements[28]. In addition cryotherapy reduces arterial blood flow, and blood uptake measured by technetium scans in healthy knees[29]. Thus by reducing tissue metabolism cryotherapy might mitigate the trauma-induced effects of ischemia, consequently reducing inflammation and pain[30].

In the upper leg, the femoral artery and several metabolically active muscles surrounding the hip joint produce a considerable amount of heat. In our study the pre-set temperature corresponded with a temperature administration of 4°C to the skin. Taking into account that temperature declines to 23°C at high pressure and low temperature setting at the mid thigh region during cryotherapy treatment, and that penetration depth of cryotherapy declines rapidly with increasing thickness of the subcutaneous fat layer, and considering the femoral artery as heat source, it is questionable if temperature reductions of clinical relevance are achieved in or near the femoral neck and its surrounding injured muscles[26]. Currently our group is developing a mathematical model that incorporates the femoral artery as heat source as well as the overlying soft-tissue layer in order to predict whether cryotherapy reduces intramuscular and bone temperature to a relevant degree.

It may be questioned if CFCT influences callus formation after fixation of the hip fracture. After fracture fixation callus formation is warranted; however little is known about the effect of cryotherapy on bone repair. Cryotherapy decreases the vasculogenic marker *VEGF-165* gene and protein expression, but does not affect differentiation, or apoptosis of human mesenchymal stem cells cultured under hypoxia[31]. This suggests that cryotherapy is not likely to harm early stages of callus formation. We found comparable callus formation between the control and intervention group.

In our study average pre treatment pooled NRS pain at rest for controls was 2.62 and for intervention subjects 2.99. Others have demonstrated that hip fracture patients, while receiving continuous epidural analgesia, almost uniformly report no postoperative pain at rest based on a 5-point scale, and that dynamic pain is higher in all types of surgery, but predominantly in the IMHN and DHS groups[2]. As mentioned by the authors, the higher dynamic pain scores might be caused by fracture micro motion. We agree on this hypothesis, hence we chose not to measure dynamic pain since, as mentioned above; it is not clearly established that CFCT will affect the deeper regions where pain from fracture micro motion might originate. Ultimately pain during specific functions (e.g. walking) is of interest but it was not considered feasible to measure dynamic pain during or shortly after CFCT in our study population.

Continuous-flow cryocompression therapy was valued 7.1 out of 10 points in our study. Post surgical anxiety is a known risk factor for the development of persistent postsurgical pain[32]. Hence increasing patient satisfaction during admission might contribute to patient' recovery and subjective pain perception, and thus can be an important pillar of the multimodal pain management strategy.

Some care has to be taken when interpreting our findings. A multicentre design is ideal for translational research since results can be applied to most patients in daily care. However the high number of study personnel at the study sites resulted in a high number of learning curves. Although a high number of nurses received training this may explain the high drop out rate and moderate protocol adherence. The variance in pain perception is large, and may explain the high standard deviation. Anxiety, chronicity or illness perception are factors that have a great influence on postoperative pain intensity[32]. Hip fracture patients often regard admission in general as stressful; combined with the burden that study participation encompasses it might cause an additional anxiety stressor, that might influence postoperative pain intensity and obfuscate a treatment effect of cryotherapy. The LOCF method might give an underestimation of the decline in NRS pain. It is known that subcutaneous fat does reduce

temperature reductions by cryotherapy[25]. Therefore a correction was made for BMI, but we did not measure thigh skin folds. A longer treatment interval could increase tissue temperature reductions, however we chose not to surpass the 30-minute treatment length in order not to hinder daily care for patients.

All cited studies apply cryotherapy in a different way: duration, frequency, type of machine (continuous-flow or not), use of a compression adjunct (static or dynamic), and temperature setting vary to some degree. These inconsistencies point out that no derivative exists to guide optimal treatment. Current recommendations are mostly based on old data and expert opinions that are based on clinical outcomes[11]. Since each treatment locus on the body has unique characteristics this derivative may vary. If we want to increase a supposed effect of cryotherapy we need to better understand of the physiological effects of cooling, and consequently develop a clear derivative on how to optimize treatment efficacy.

In conclusion, no evidence was recorded to suggest that CFCT has an added value in the acute recovery phase after hip fracture surgery. However CFCT has mild analgesic effects in patients that complete CFCT treatment during the first 72 h. In an attempt to optimize CFCT treatment, a better understanding of the physiological effects of cooling by cryotherapy machines and its efficacy in various (obese) body constitutions should be sought.

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Chapter 6

Continuous-flow cryocompression therapy penetrates to bone leven in hip fracture patients in a numerical simulation

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Abstract

Background: Cryotherapy is used to alleviate pain after musculoskeletal trauma, even though hypothermia might retard bone healing. Whether cryotherapy reduces temperature at the bone level after hip fracture surgery, possibly leading to impaired bone healing is unknown. We aimed to define deep tissue temperature during cryotherapy in postoperative hip fracture patients, by using measured skin temperature as input parameter for a simple numerical model. Second, an association was investigated between pain and tissue temperature distribution, to assess cryotherapy-induced analgesia of soft tissue derived pain.

Methods: Data were retrieved from 35 subjects who participated in an ongoing trial. In three subjects who consented on additional measurements skin temperature was measured in three days during and after cryotherapy. A simple numerical model was developed to calculate tissue temperature distribution during cryotherapy.

Results: Inter and intra subject skin temperature displayed high variation: trochanter 11-27°C, mid-femur 11-24°C, distal femur 10-16°C. Predicted temperatures decreased to 20°C at 1 cm, 26°C at 2 cm, and 30°C at 3 cm tissue depth. Smallest soft tissue layer measured at the trochanter; 42% had less than 30 mm and 21% had less than 20 mm. NRS pain varied (mean=2.14; SD=1.92), no association was found between pain and decrease in temperature (r=0.064; p=0.204).

Interpretation: Cryotherapy reduced temperature up to 3 cm, in cachectic patients this reached the bone, where it might have implications for bone tissue healing when treated for a prolonged period of time. Cryotherapy-induced analgesia is likely to originate from skin analgesia rather than analgesia of muscle or bone derived pain.

Introduction

Cryotherapy is used to treat pain after musculoskeletal trauma such as total hip arthroplasty, total knee arthroplasty and hip fractures (Bugaj 1975, Adie et al. 2010, Song et al. 2016, Leegwater et al. 2017). Skin temperature of less than 13.6°C produces skin analgesia (Bugaj 1975). In ankles of healthy subjects, tibial nerve conduction velocity is reduced with 33% at 10°C skin temperature, resulting in a higher pain threshold (Algafly and George 2007). Continuous-flow cryocompression therapy (CFCT) reduces skin temperature to 10°C, and to 22-25°C up to 1.5 cm below the subcutaneous layer in thighs of healthy individuals (Holwerda et al. 2013). Cryotherapy diminishes the cell's metabolic rate of glucose, oxygen, and lactate production by 2 to 4-fold per 10°C drop in the mammalian central nervous system (Erecinska et al. 2003). Mild hypothermia (28–34°C) has shown to produce anti-inflammatory effects in healthy subjects (Lubkowska et al. 2011), but sub-physiologic temperatures of less than 32°C are also known to severely decrease proliferation in mammalian cells (Fujita 1999), and halts proliferation completely in mouse embryo fibroblasts (Wilke and Weiner 2003). In addition, 34°C hypothermia severely impairs osteoblast activity, and doubles osteoclast activity in a calvarial rodent model (Patel et al. 2012). Thus a decrease in skin temperature is advantageous in order to treat pain, but in the posttraumatic recovery phase, an increase in tissue metabolism is warranted in order to ensure adequate fibroblast, osteoblast and osteoclast function for repair of soft tissue and bone. Whether cryotherapy reduces temperature at the bone level after hip fracture surgery, possibly leading to impaired cellular function is unknown. Furthermore it is also unclear whether cooling of muscle and bone tissue at greater depth contributes to the analgesic effect of cryotherapy.

The primary aim of this study was to define deep tissue temperature during CFCT in postoperative hip fracture patients, by using measured skin temperature as input parameter for a simple numerical model. Second, an association between tissue temperature distribution and pain was investigated to assess cryotherapy-induced analgesia of soft tissue derived pain. We hypothesized that: 1) soft tissue temperature at the bone level (as determined on X-ray) will decrease during cryotherapy and 2) that temperature decrease and decline in pain perception by hip fracture patients as measured by numeric rating scale (NRS) pain are associated.

Methods

After obtaining written informed consent, data on demographics, height, weight, implant type, and pain scores before and after CFCT were obtained from a subset of 35 subjects who were included in a multi-center randomized controlled trial to assess the effects of CFCT (Leegwater et al. 2017). All subjects that received CFCT at the Spaarne Gasthuis hospital were selected.

The CFCT was applied by using the 'Game Ready System' (GRS; CoolSystems Alameda, California) according to the cryotherapy treatment protocol as published earlier (Leegwater et al. 2016). Through an anatomically designed hip/groin wrap covering most of the thigh and pelvis up to the iliac crest, the GRS simultaneously delivers both adjustable continuous-flow cryotherapy and intermittent compression. The machine has four pressure settings: no pressure, low-pressure (5-15 mm Hg), medium-pressure (5-50 mm Hg) and high-pressure (5-75 mm Hg). Temperature can be adjusted between 4°C and 13°C, the lowest temperature was used. Pressure started at 'low' and increased stepwise per 4 treatments. Treatment cycles were administered 4 times daily during the first 72 postoperative hours. A sole investigator applied and/or removed the wrap to perform the temperature measurements to reduce measurement variability. Before and after each treatment cycle the subject was asked to verbalize perceived NRS pain.

In subjects who consented with additional temperature measurements the cooling area was measured with a 4-channel thermometer type TM-947SD (Lutron Electronic Enterprise Co., Taipei, Taiwan) with thermo probes type T; 12M-T-0.5 Class 1 (Lutron Electronic Enterprise Co.) during and after cryotherapy treatment on three consecutive evenings. Probes were placed laterally at: the major trochanter level, mid femur level, the distal femur level 5 cm proximal to the upper margin of the patella, and laterally on the contralateral femur. After thermo probe placement CFCT was administered for 30 minutes in the evening. Upon completion the wrap was removed and the subject was covered in normal blankets. Subjects remained in bed overnight until the thermo probes were detached the following morning. Skin temperature was measured every 10 seconds. For total hip arthroplasty, hemiarthroplasty and dynamic hip screw the mid femur temperature was used as input skin temperature for the model because most soft tissue trauma is present at this level, whereas the proximal skin temperature was used for the intramedullary hip nail since most soft tissue trauma is present at the entry point of the nail.

A simple validated numerical model was used to calculate tissue temperature during CFCT. The model uses a finite difference method to solve a one-dimensional temperature equation

with the Crank-Nicolson scheme (Recktenwald 2004). In order to solve the equation $\Phi(x,t)$ where Φ is the temperature, 'x' is the skin depth and 't' is time, the following three boundary conditions were used. First, the temperature at the skin (x=0) equals the aforementioned measured skin temperature data ($\Phi(0,t)=T_{meas}(t)$). Second, the temperature at maximum depth 'L' of 10 cm depth (x=L) equals the measured core temperature of the subject (T_0) before CFCT ($\Phi(L,t)=T_0$). Finally, the initial temperature of the simulated body was also set to the measured core temperature of the subject before CFCT ($\Phi(x,t_0)=T_0$). The body mass index (BMI) was calculated to determine the overall body mass. BMI and body fat percentage (BF%) are correlated and based on this correlation BF% was calculated from the BMI, and used as input parameter (Camhi et al. 2011). Assuming the tissue to be a homogeneous fat-water mixture, the thermal diffusivity was linearly interpolated between plain water ($0.149*10^{-6}$ m^2/s) and fat ($0.1*10^{-6}$ m^2/s), based on the BF% of the subject. A one-second-temperature resolution during a simulated time of half an hour, and a spatial resolution of 0.1 mm were used.

Soft tissue dimensions were obtained from X-rays of all subjects (Figure 1). The measurements were calibrated using implanted osteosyntheses with known dimensions. With the numerical simulation model, the minimum and mean temperature at those depths during CFCT was determined.

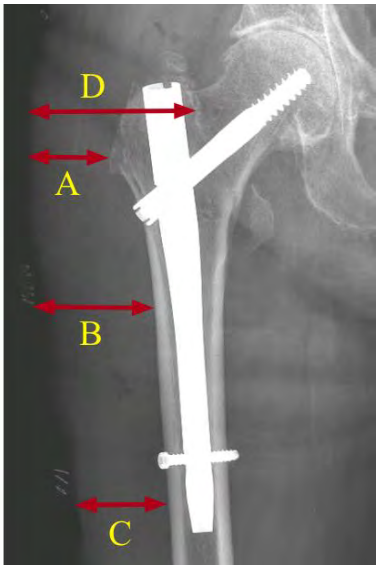
Statistics

Statistical analysis was performed by use of IBM SPSS Statistics for Windows, Version 25 (IBM Corp. Released 2013. Armonk, NY: IBM Corp.). Subject and clinical characteristics were described according to their distribution. Continuous data were described as means with ranges in case of normality. Categorical data were presented as frequencies with accompanying percentages. Temperature measurements for each of the three subjects are presented as absolute values, the simulated soft tissue temperature of all subjects was presented as mean with standard deviation (SD) for each dimension.

Both univariate and multivariate analyses were performed to assess the association between temperature distribution after CFCT and treatment effect during the first 72 postoperative hours. Treatment effect was defined as NRS pain improvement, which is defined as pre treatment NRS pain minus post treatment NRS pain. Mixed-model repeated-measures analysis of covariance was performed and adjusted for potential confounders (gender, body-mass index (BMI), surgery type, fracture type, and pressure settings).

Additional analysis was performed to assess the course of NRS pain after treatment during the first 72 hours. A p-value < 0.05 was considered statistically significant.

Figure 1. Tissue dimensions measured on a postoperative X-ray of an implanted IMHN.



A: trochanter distance, B: mid femur distance, C: distal femur distance, D: shortest skin-to-fracture distance

Ethics, funding, and potential conflicts of interest

The Medical Ethical Committee 'METC Noord-Holland', Alkmaar, The Netherlands (date: October 13, 2015; reference no: NH015.188) approved amendments in an ongoing trial (Leegwater et al. 2016) that enabled additional measurements to be performed for the current study. No funding was acquired for the study. The authors declare no conflict of interest.

Results

Data from 35 subjects was used; subject characteristics are displayed in Table 1. In 10 subjects incomplete pain measurements precluded pain analysis. Post treatment NRS pain declined over the course of treatments during 72 h with 0.14 NRS per 6 h from 3.0 to 1.57 (p <0.001).

Table 1. Subject characteristics.

Variable	**Cryotherapy subjects** (n=35)
Female, *n*	26
Age, years	80.7 (10.4)
Weight, kg	66.8 (15.8)
Height, cm	168.3 (10.7)
BMI, kg/m^2	24.1 (4.1)
Type of surgery, *n*	
- DHS	9
- HA/THA	12
- IMHN	14

Data are reported as mean and standard deviation unless stated otherwise. BMI: body mass index; DHS: dynamic hip screw; HA: hemiarthroplasty; THA: total hip arthroplasty; IMHN: intramedullary hip nail.

Two males and one female consented with skin temperature measurements. Subject one and two both had a peritrochanteric fracture and an intramedullary hip nail was implanted, subject one was a cachectic male (age 76 years, weight 62 kg, height 182 cm), and subject two was an obese male (age 93 years, weight 100 kg, height 175 cm). Subject three was a cachectic female (age 91 years, weight 53 kg, height 150 cm) with a medial column fracture where a hemiarthroplasty was performed. In one subject only two registrations instead of three sets were obtained. In one registration cycle, the distal temperature probe registered a minimum temperature of 30.8°C, this measurement was considered an error (probe was not covered by the wrap) and was omitted from further data analysis. This resulted in a total of eight registration cycles, and in one of these cycles only two thermo probes provided viable data (Table 2). Inter and intra subject skin temperature varied greatly (Table 2). In subject 2 the high-pressure setting resulted in the lowest skin temperature measured, while in subject 3 the high-pressure setting did not result in a decrease in skin temperature. In all cases the fastest decrease in skin temperature was observed within the first five minutes of treatment, and at the end of CFCT a minimum temperature of 9.9°C was reached at mid femur (Figure 2). After cessation of CFCT, it took 5.5 minutes before the temperature rose above 13.6°C,

and after 179 minutes (SD 52.7) the baseline temperature was reached (Figure 2). No reactive hyperthermia was observed after cessation of CFCT (Figure 2).

Table 2. Skin temperature measurements during cryotherapy treatment.

				Minimum skin temperature (°C)			
Subject	**BMI[a]**	**Timing**	**Pressure**	**Trochanter**	**Mid femur**	**Distal femur**	**Contralateral**
1	18.7	POD 1	Low	-	-	-	-
		POD 2	High[b]	21	-	-	34
		POD 3	High	18	11.5	14	36
2	32.6	POD 1	Low	27	20	16	34.5
		POD 2	Medium	12	19	16	34
		POD 3	High	10.5	20.5	31	34
3	23.6	POD 1	Low	13	18.5	10	31
		POD 2	Medium	24	24	15	33.5
		POD 3	High	26	11.5	14.5	34.5

POD: postoperative day. [a] kg/m^2, [b] Pressure was incorrectly set to high-pressure at POD 2.

In model simulations, after CFCT the average temperature dropped from an initial temperature of 32.1°C to 18.2°C at the skin, 24.1°C at 1 cm tissue depth, 28.1°C at 2 cm tissue depth, and 30.4°C at 3 cm tissue depth (Figure 3). Temperature did only slightly drop at 3 cm tissue depth, and was unchanged in depths below 3 cm (Figure 3). Soft tissue dimensions were acquired from X-rays in 24 subjects, in four subjects no X-rays were taken, and in seven subjects insufficient X-ray quality precluded measurements. Forty-two percent had a skin-to-bone distance of less than 30 mm, and 21% had a distance of 20 mm or less, the smallest distance was usually measured at the trochanter level. The lowest temperatures were observed at the trochanter and the distal femur (Table 3).

No association was found between NRS pain and tissue temperature distribution at the central soft tissue (adjusted β-coefficient 0.03 (-0.24; 0.31) p=0.81)).

Table 3. Calculated deep soft tissue temperature after cryotherapy treatment.

		Simulated temperature (°C) at X-ray distance		
Dimension	**X-ray[a]** (mm)	**Minimum**	**Average**	**Maximal**
A – Trochanter	34.1 (13.8)	27.1 (2.7)	30.1 (1.9)	31.9 (1.3)
B – Central	42.6 (13.6)	31.7 (1.7)	32.5 (1.1)	33.0 (0.7)
C – Distal	37.6 (11.6)	31.0 (2.0)	32.0 (1.3)	32.8 (0.8)
D – Fracture distance[b]	59.6 (10.8)	32.8 (2.1)	33.1 (1.3)	33.3 (0.8)

Data are reported as mean and standard deviation. n=35. [a] Skin distance based on first postoperative X-ray from 24 subjects. [b] The shortest distance measured from skin to the center of the fracture, or the lateral confinement of the prosthesis in THA/HA subjects.

Figure 2. Temperature drop during 30 minutes of continuous-flow cryocompression therapy, and subsequent passive rewarming.

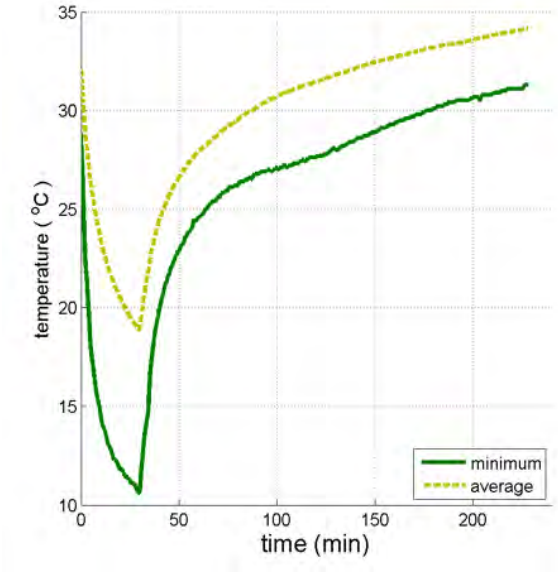
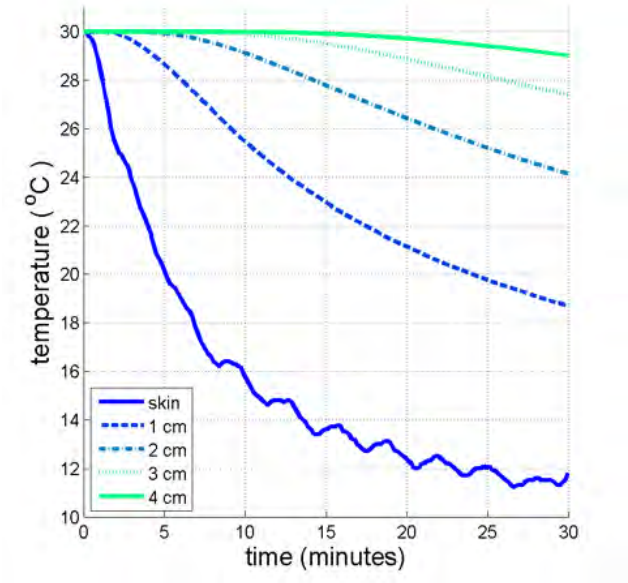


Figure 3. Calculated temperature distribution during continuous-flow cryocompression therapy at various tissue depths.



Discussion

Cryotherapy is used to alleviate pain and to reduce inflammation after musculoskeletal trauma (Schaser et al. 2006, Song et al. 2016, Leegwater et al. 2017), but hypothermia might not always be beneficial for various cell types that are required for adequate healing of soft tissue and bone. It is important to understand to which depth CFCT reduces temperature in order to put the effects on cellular function that are already known into perspective. Also a correlation between tissue temperature distribution and pain perception might help to address the knowledge gap whether cooling of muscle and bone tissue at greater depth contributes to the analgesic effect of cryotherapy.

This is the first study attempting to define CFCT-induced tissue temperature drop, and attempting to correlate this decline with a change in patient-reported pain in postoperative hip fracture patients. We found the minimum skin temperature to drop to 9.9°C at the distal femur level, 11°C at mid femur and trochanter level by cryotherapy. These observations are in line with others who measured a minimum skin temperature of 12°C at 1.5 cm below the skin at the distal thigh of healthy individuals with the same cryotherapy machine that was used in the current study (Holwerda et al. 2013). Skin temperature drops to 10°C when an ice pack or when a 4:1 water – alcohol (70%) mixture is applied (Kanlayanaphotporn and Janwantanakul 2005). The pressure setting did not affect cooling efficacy at the skin level in our obese subject, but it did augment cooling in our cachectic subject. External elastic compression augments cooling efficacy of ice bag treatment (Tomchuk et al. 2010). In CFCT, increments in the dynamic pressure setting also allows for a more effective cooling regimen (Holwerda et al. 2013). Although dynamic compression increases CFCT's efficacy, it does not surpass the more traditional solid-state ice application modalities in skin temperature reduction. Heterogeneity in application and tightening of the GRS wrap can be an explanation for the varying skin temperatures we measured within our subjects. In addition, subjects with a high BMI have excess fat around the hips that forms the upper leg in a cone-like shape, having the greatest circumference proximally as opposed to a small circumference distally. This shape may cause difficulty in properly conforming the wrap to the thigh; the wrap usually covers most of the upper leg, as opposed to solid-state ice bags that are applied directly at the target area.

In our model calculations, temperature dropped to 23°C at 1.5 cm and to 26°C at 2 cm tissue depth. These findings are in line with others who measured temperature drop by cryotherapy at these depths (Tomchuk et al. 2010, Holwerda et al. 2013). Ice bag treatment combined with an elastic wrap reduces temperature to 25°C at 2 cm in 30 minutes (Tomchuk

et al. 2010), whereas the cryotherapy device that was used in the current study reduces soft tissue to 23°C at 1.5 cm depth at the high-pressure setting (Holwerda et al. 2013). We found no reports on temperature drop at deeper levels. In our model a temperature drop from 32.1°C to 30.4°C at 3 cm was the deepest level where a significant drop in temperature was calculated. Forty-two percent of the subjects had a skin-to-bone distance of less than 30 mm, and 21% had a distance of 20 mm or less at the major trochanter (the entry-point for IMHN). This suggests that CFCT not only reduces soft tissue temperature but might also reduce bone temperature in hip fracture patients that have less than 30 mm of soft tissue. Temperatures such as 25-30°C may retard connective tissue healing since proliferation is severely decreased (Fujita 1999), and proliferation halted in mouse embryo fibroblasts at 32°C (Wilke and Weiner 2003). It might also have implications for bone healing, as osteoblast activity is severely impaired and osteoclast activity promoted in murine calvarial cells subjected to 34°C (Patel et al. 2012). We previously determined that hypothermia reduces *VEGF-165* protein expression (a marker of vasculogenesis) under hypoxia, but we did not find osteogenic differentiation of human adipose stem cells to be impaired (Leegwater et al. 2017). Since the CFCT treatments in our study were applied intermittently and only for 30 minutes, the temperature drop is short-lived. However if applied continuously and for a prolonged period of time CFCT might adversely affect connective and bone tissue healing.

Continuous-flow cryocompression therapy reduces pain in subjects that completed the 3-day treatment schedule in our multicenter study (Leegwater et al. 2017). In the current study we attempted to determine an association between a predicted tissue temperature drop and a decline in NRS pain from these subjects. However we found no association between tissue temperature and pain perception. Two pathways can be proposed on how cryotherapy exerts its analgesic efficacy, either deeply via an interaction with tissue metabolism and immunomodulation, or superficially via an interaction on nerve conduction. After induced soft tissue trauma, cryotherapy restores microcirculatory hemodynamics in rats (Schaser et al. 2006). Cryotherapy increases the level of anti-inflammatory cytokines IL-6 and IL-10, which are known to be related to pain (Zhou et al. 2016, Alvarez et al. 2017), and decreases the pro-inflammatory Il-1 α cytokine level in humans (Lubkowska et al. 2011). In light of the superficial pathway, others determined skin temperatures of less than 13.6°C to produce skin analgesia (Bugaj 1975), and demonstrated reduced nerve conduction velocity due to decreased skin temperature (Algafly and George 2007). The reduced nerve conduction velocity correlated with an increase in pain threshold, the threshold was measured with a pressure algometer in the ankles of healthy subjects (Algafly and George 2007). Objectively

and reliably assessing patient-reported pain perception remains difficult, and often standardized pain assessment methods are used to more accurately assess pain. However these results are difficult to translate to the clinical setting. It is questionable if this increase in pain threshold that is measured on the skin indeed provides a clinical noticeable analgesic effect in a postoperative setting, since postoperative pain originates from more than only skin trauma. In our subjects skin temperature dropped below 13.6°C to 9.9°C, which is sufficient to produce skin analgesia (Bugaj 1975, Algafly and George 2007). The reduction of 1.5 NRS pain that was observed in our multicenter study (Leegwater et al. 2017) might suggest that skin analgesia is present. However the high variation in NRS pain and skin temperature we measured does hamper sound conclusions about whether the remaining pain originates from muscular tissue or the periost.

Some carefulness has to be taken when interpreting our findings. Post implantation fever might confound CFCT cooling efficacy. Although no temperature fluctuations were measured in the contralateral leg that might illustrate post implantation fever, it cannot be ruled out since this, when transient, is not always accompanied by increase in skin temperature. We did not measure skin folds although subjects with greater skinfold thickness required longer cryotherapy application time in order to produce similar tissue temperature changes (Otte et al. 2002). Instead we calculated the BF% from the BMI. The measured skin distances on the postoperative X-ray that were used for the input parameter in the model are static, although edema or hematoma is not likely to dissipate within the first 72 hours it might be influenced by the therapy itself. Our model uses flat plate geometry, the simulated temperature drop at deeper distances will be underestimated. In reality on the other hand, even though the therapy applies an external pressure to the tissue, the tissue will be perfused with a constant supply of warm blood. As this is not incorporated in the model, the model overestimates the temperature decrease. However, these effects act in opposite direction. With our simulation we also found similar temperature and a similar penetration depths of 2-3 cm during 30 minutes CFCT, which matches to temperatures and penetration depths found in literature (Tomchuk et al. 2010, Holwerda et al. 2013). This suggests the simple model is adequate for our research questions.

In conclusion, cryotherapy reduced temperature up to approximately 3 cm tissue depth, in cachectic patients this reached the bone, where it might have implications for bone tissue healing when treated for a prolonged period of time. The lack of a correlation between pain and predicted decrease in temperature might implicate that cryotherapy-induced analgesia is likely to originate from skin analgesia rather than analgesia of muscle or bone derived pain.

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Chapter 7

Hypothermia reduces VEGF-165 expression, but not osteogenic differentiation of human adipose stem cells under hypoxia

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Abstract

Cryotherapy is successfully used in the clinic to reduce pain and inflammation after musculoskeletal damage, and might prevent secondary tissue damage under the prevalent hypoxic conditions. Whether cryotherapy reduces mesenchymal stem cell (MSC) number and differentiation under hypoxic conditions, causing impaired callus formation is unknown. We aimed to determine whether hypothermia modulates proliferation, apoptosis, nitric oxide production, VEGF gene and protein expression, and osteogenic/chondrogenic differentiation of human MSCs *under hypoxia*. Human adipose MSCs were cultured under hypoxia (37°C, 1% O_2), hypothermia and hypoxia (30°C, 1% O_2), or control conditions (37°C, 20% O_2). Total DNA, protein, nitric oxide production, alkaline phosphatase activity, gene expression, and VEGF protein concentration were measured up to day 8. Hypoxia enhanced *KI67* expression at day 4. The combination of hypothermia and hypoxia further enhanced *KI67* gene expression compared to hypoxia alone, but was unable to prevent the 1.2-fold reduction in DNA amount caused by hypoxia at day 4. Addition of hypothermia to hypoxic cells did not alter the effect of hypoxia alone on *BAX*-to-*BCL-2* ratio, alkaline phosphatase activity, gene expression of *SOX9*, *COL1*, or osteocalcin, or nitric oxide production. Hypothermia decreased the stimulating effect of hypoxia on *VEGF-165* gene expression by 6-fold at day 4 and by 2-fold at day 8. Hypothermia also decreased VEGF protein expression under hypoxia by 2.9-fold at day 8. In conclusion, hypothermia decreased *VEGF-165* gene and protein expression, but did not affect differentiation, or apoptosis of MSCs cultured under hypoxia. These *in vitro* results implicate that hypothermia treatment *in vivo*, applied to alleviate pain and inflammation, is not likely to harm early stages of callus formation.

Introduction

Fractures are generally accompanied by soft tissue trauma that is aggravated by subsequent surgical stabilization. The interruption of arterial vascular flow causes regional ischaemia and hypoxia, resulting in inflammation [1]. Cryotherapy seems to be a modulator of the posttraumatic inflammatory reaction, but results obtained do not unanimously agree on the way it affects inflammation. Cryotherapy has been reported to reduce posttraumatic microvascular dysfunction, inflammation, and structural impairment in a rodent model [2]. However, hypothermia prolongs the inflammatory response systemically and locally in fracture hematomas in a porcine model [3]. Cryotherapy can be applied in the acute recovery phase of musculoskeletal trauma and after orthopaedic surgical interventions, such as knee arthroplasty to prevent pain and inflammation [4]. Currently, the clinical effect of cryotherapy is being investigated in postoperative hip fracture patients [5], even though the effect of application of cryotherapy on osteoblast precursor proliferation and differentiation during bone tissue repair has not been clearly established.

During the process of bone repair, the interruption of vascular flow activates the coagulation and formation of a fracture hematoma, which has a remarkable angiogenic capacity [1]. Moreover, hypoxia is a key factor in bone repair [1]. It increases human mesenchymal stem cell (MSC) migration rates and improves their tissue regenerative potential in a murine hind limb ischaemia model [6]. Furthermore hypoxia induces recruitment of fibroblasts and osteogenic progenitor cells via the production of reactive oxygen species (ROS) [7,8]. At *low* concentrations, ROS functions as a messenger to enhance wound healing [9]. It targets survival pathways such as mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)/Akt [8,10]. ROS also targets hypoxia-inducible factor-1 (HIF-1), which upregulates gene expression of vascular endothelial growth factor (VEGF), an angiogenesis and vasculogenesis-inducing agent as well as a bone-metabolism cytokine that stimulates the differentiation and chemotactic migration of osteoblast precursor cells [11–13]. However, hypoxia is not always beneficial for bone repair. Physiological ROS formation is disrupted during ischaemia and subsequent reperfusion [14]. Pathological hypoxia sustained during ischaemia causes ROS accumulation [14], which has been implicated in secondary tissue damage. Cooling of ischaemic tissues using cryotherapy, which decreases muscle temperature to 23°C at the thigh of healthy individuals [15], might overcome some of the adverse effects of the pathological hypoxic state and excessive ROS formation. However, the effect of cryotherapy on cell metabolism in fracture haematomas is currently unknown.

Cryotherapy diminishes the cell's metabolic rate of glucose, oxygen, and lactate production by 2 to 4-fold per 10°C reduction in the mammalian central nervous system [16]. Synovial lactate concentrations remain stable despite a decrease in blood flow (indicated by increased ethanol exchange ratio) when cryotherapy is applied in patients recovering from arthroscopy, suggesting a decrease in energy requirements [17]. A reduction in ROS concentration by hypothermia has been shown to attenuate the apoptotic cascade in murine nerve cells [18]. Thus, cryotherapy likely reduces cell metabolism in haematomas of fracture patients, thereby it might reduce harmful concentrations of ROS. In addition, hypothermia blocks ß-catenin degradation via the PI3K/Akt pathway in a focal ischaemic rat model, resulting in decreased cell injury and apoptosis [19], and activation of the PI3K/Akt pathway is suggested to enhance osteogenic differentiation of MSCs [20].

On the other hand, hypothermia reduces osteoblast proliferation and differentiation while promoting osteoclast function in cultured rat calvariae [21]. Taken together, these findings warrant further investigation of induced hypothermia effects on early stages of bone healing. To date no studies have addressed whether hypothermia affects osteogenic differentiation and proliferation of MSCs, be it in a positive or negative way, under hypoxic conditions.

We aimed to determine whether hypothermia modulates proliferation, apoptosis, nitric oxide (NO) production, VEGF gene and protein expression, and osteogenic/chondrogenic differentiation of human mesenchymal stem cells *under hypoxia*. We hypothesized that hypoxia stimulates osteogenic/chondrogenic differentiation, VEGF gene and protein expression, NO production, and apoptosis, and inhibits MSC proliferation, but that hypothermia attenuates these hypoxia-induced effects.

Materials and Methods

Donors

hASCs were isolated from abdominal subcutaneous adipose tissue as waste material after abdominoplasty from six Caucasian healthy female donors (age 31-56) at Tergooi Hospital, Hilversum, The Netherlands. Our study has been conducted within the framework of the "Medical Research Involving Human Subjects Act (WMO) exemption" as ruled by the Medical Ethical Committee of the VU University Medical Centre, Amsterdam, The Netherlands (date: 17-03-2016; reference no: 2016.105). The use of all human materials in this study has been approved by the Medical Ethical Committee of the VU University

Medical Centre ("Medisch Ethische Toetsingscommissie VU medisch centrum"; protocol number 2005/128) after obtaining written informed consent.

hASCs isolation and culture

Human adipose tissue obtained by resection was stored in sterile phosphate buffered saline (PBS) at 4ºC overnight and processed within 24 h, as described previously [22,23]. In brief, adipose tissue was minced, washed with PBS, and enzymatically digested with 0.1% collagenase A (Roche Diagnostics, Mannheim, Germany) in PBS containing 1% bovine serum albumin (BSA; Roche Diagnostics). The resulting cell pellet containing the hASCs was resuspended, and viability and cell number was measured with a NucleoCounter® (NC-100™, ChemoMetec, Allerod, Denmark). Confirmation of stem cell phenotype has been confirmed earlier by surface marker expression [22,23]. The attached ASCs are virtually all positive for markers CD29 (cell adhesion marker), CD73, CD90, and CD105 (MSC-associated markers), CD166 and HLA-ABC (leucocyte surface markers), while they do not express leucocyte surface markers CD45 or HLA-DR [22,23]. For cell culture, single cell suspensions of cryopreserved hASCs were thawed and seeded at 4-12x10^4 cells/cm^2 in α-Modified Eagle's Medium (α-MEM; Gibco, Paisley, UK) supplemented with 5% platelet lysate (PL; VU University Medical Centre, Amsterdam, The Netherlands), 0.2% (vol/vol) heparin (5,000 U/ml), and 1% antibiotic-antimycotic solution (10,000 U/ml penicillin (Gibco), 10 mg/ml streptomycin (Gibco), and 25 µg/ml amphotericin B (Sigma)), and cultured under 5% CO_2 and 20% O_2, at 37°C. Platelet lysate is known to induce osteogenic differentiation of hASCs [24,25]. Upon reaching 80-90% confluency, cells were harvested by incubation with 0.25% trypsin/0.1% ethylenediaminetetraacetic acid (EDTA; Gibco) in PBS for 5 min at 37ºC. All cells used were from passage 4 or less. For experiments, hASCs were seeded at 10,000 cells/cm^2 in 6-well dishes containing α-MEM supplemented with 2% PL, 0.2% heparin (5,000 U/ml), and 1% antibiotic/antimycotic solution, and cultured under hypoxia (37°C, 1% O_2), the combination of hypothermia and hypoxia (30°C, 1% O_2), or control conditions (37°C, 20% O_2) for 1, 4, and 8 days in α-MEM with supplements, with medium refreshment at day 4. At day 1, 4, and 8, cells were lysed for total RNA isolation, and quantification of total DNA, protein content, and alkaline phosphatase (ALP) activity as described below. We have chosen our time points based on earlier findings that hASCs show changes in proliferation, gene expression of KI67, COL1, and osteocalcin, and changes in ALP activity at 48 h, 4 days [26,27]. Moreover, since platelet lysate is a strong inducer of osteogenic differentiation in hASCs, we obtained statistical significant differences already at relatively early time points.

Culture under hypoxia

For culturing in hypoxia, hASCs were placed in a NAPCO® incubator (serial number 7101-C1, Precision Scientific Inc., Chicago, IL), in which the oxygen concentration is controlled by flushing with N_2. Oxygen levels in the incubator were monitored by an internal oxygen sensor, as well as by external calibration using Dräger Tubes 6728081 (Drägerwerk Ag, Lübeck, Germany). Hypoxia was defined as 1% O_2/5% CO_2 in air.

RNA isolation and gene expression analysis

Cells were washed with PBS and lysed with 700 µl TRIzol® reagent (Life Technologies, Carlsbad, CA). Total RNA was isolated according to the manufacturer's instructions. cDNA synthesis was performed using 750 ng of total RNA with reaction mixture Transcriptor First Strand cDNA synthesis kit (Roche Diagnostics, Mannheim, Germany), in an Applied Biosystems® GeneAmp® PCR System 9700, creating 20 µl suspension.

Real-time polymerase chain reaction (PCR) was used to determine gene expression of the osteogenic markers osteocalcin, collagen type 1 (*COL1*), and the chondrogenic marker *SOX9*. *KI67* gene expression was measured as proliferation marker. The ratio of *BAX*-to-*BCL-2* gene expression was used as an indicator of cell apoptosis. *VEGF-165* gene expression was measured as a marker of vasculogenesis. Three housekeeping genes (*TBP, HPRT,* and *YWHAZ*; InVitrogen, Carlsbad, CA) were used to correct for the combined effect of hypothermia and hypoxia. Real-time PCR reactions were performed with 1 µl cDNA (5x dilution) and ready to use hot start master mix LightCycler® 480 SYBR Green I Master (Roche Diagnostics) in a LightCycler® 480 Real-Time PCR System (Roche Diagnostics). The primer sequences are listed in Table 1.

Total DNA, protein content, and ALP activity

hASCs were lysed with 0.7 ml of ice-cold Milli-Q water, harvested on ice, sonicated for 10 min in ice-cold water, and centrifuged for 10 min at 2,000 rpm at 4ºC. The supernatants were immediately analysed for total DNA, protein content, VEGF protein concentration (see below), and ALP activity. Total DNA was quantified using the CyQUANT® Cell Proliferation Assay (Molecular Probes, Eugene, OR) according to the manufacturer's protocol. ALP activity was measured in the supernatant according to the method described by Lowry [28]. Total protein was determined using a BCA Protein Assay Reagent kit (Pierce, Rockford, IL). ALP activity and protein content were normalized for DNA. Absorbance was measured

with a SynergyTM HT (BioTek Instruments, Winooski, VT) microplate reader in concordance with the manufacturer's instructions.

Nitric oxide

NO production was measured as nitrite (NO_2^-) accumulation in the conditioned medium (CM) using Griess reagent containing 1% sulfanilamide, 0.1% naphtylethelene-diamine-dihydrochloride, and 2.5 M H_3PO_4. Serial dilutions of $NaNO_2$ in non-CM were used as a standard curve. Measurements were performed with a SynergyTM HT microplate reader.

VEGF protein

To determine VEGF protein concentration in the supernatant, a Quantikine® ELISA kit (R&D Systems Inc., Minneapolis, MN) was used and the samples were assayed according to the manufacturer's protocol. Measurements were performed with a SynergyTM HT microplate reader.

Statistical analysis

All data were checked for normality by using the Kolmogorov Smirnoff's test. If applicable, logarithmic transformation was performed to obtain normal distributions. Mixed Model Analysis included temperature and oxygen concentration as fixed factors. The interaction between the factors was also evaluated. Statistical differences were considered significant if $p<0.05$. Post hoc multiple pairwise comparisons were performed at each time point with an adjusted significance level of 0.017 using Bonferroni's method. All data were evaluated using the IBM SPSS statistical package for Macintosh, Version 20.0 (Armonk, NY).

Table 1. Primers used in the real-time PCR assay.

Gene		Oligonucleotide Sequence	Amplicon length (bp)
TBP	Forward	5' GGTCTGGGAAAATGGTGTGC 3'	97
	Reverse	5' GCTGGAAAACCCAACTTCTG 3'	97
HPRT	Forward	5' GCTGACCTGCTGGATTACAT 3'	260
	Reverse	5' CTTGCGACCTTGACCATCT 3'	260
YWAZ	Forward	5' GATGAAGCCATTGCTGAACTTG 3'	229
	Reverse	5' CTATTTGTGGGACAGCATGGA 3'	229
Ki67	Forward	5’ GGTGGGCACCTAAGACCTGAA 3’	235
	Reverse	5’ TCCTAGGACTAGGAQGCTGGAG 3’	235
Osteocalcin	Forward	5’ AGCCACCGAGACACCATGAGA 3’	288
	Reverse	5’ CTCCTGAAAGCCGATGTGGTC 3’	288
SOX9	Forward	5’ CCACACTCCTCCTCCGGCATGA 3’	188
	Reverse	5’ TCCACGTCGCGGAAGTCGAT 3’	188
VEGF-165	Forward	5' ATCTTCAAGCCATCCTGTGTGC 3'	224
	Reverse	5' CAAGGCCCACAGGGATTTTC 3'	224
BAX	Forward	5' CACCAGCTCTGAGCAGATCAT 3'	345
	Reverse	5' CTTGGTGCACAGGGCCTTG 3'	345
BCL-2	Forward	5' GACTTCGCCGAGATGTCCAG 3'	232
	Reverse	5' AGGTGCCGGTTCAGGTACTC 3'	232
COL1	Forward	5' TCCGGCTCCTGCTCCTCTTA 3'	336
	Reverse	5' GGCCAGTGTCTCCCTTG 3'	336

Results

Hypoxia decreased total DNA at day 4 by 1.2-fold (p=0.004) compared to controls (Fig 1A). The combination of hypothermia and hypoxia further reduced total DNA in hASCs at day 1 by 1.4-fold (p=0.008), and at day 4 by 1.2-fold (p=0.013), but not at day 8, compared to hypoxia alone.

Hypoxia upregulated *KI67* gene expression at day 4 by 5-fold (p=0.0051) compared to controls (Fig 1B). The combination of hypothermia and hypoxia downregulated *KI67* gene expression at day 1 by 8.8-fold (p=0.0007), but increased *KI67* gene expression at day 4 by 15-fold (p=0.001) and at day 8 by 21-fold (p=0.002), compared to hypoxia alone (Fig 1B).

Hypoxia did not significantly affect *BAX*-to-*BCL-2* gene expression ratio. The combination of hypothermia and hypoxia decreased the *BAX*-to-*BCL-2* gene expression ratio at day 8 by 5.1-fold (p=0.016) compared to controls, but not compared to hypoxic conditions (Fig 1C).

Hypoxia reduced cell-associated ALP activity, a marker of osteogenic differentiation, at day 8 by 1.9-fold (p=0.015) compared to control hASCs (Fig 2A). The combination of hypothermia and hypoxia reduced cell-associated ALP activity at day 8 by 4-fold (p=0.016) compared to control MSCs, but no effect of hypothermia under hypoxia was found compared to hypoxic conditions. Hypoxia nor the combination of hypothermia and hypoxia affected the gene expression of osteogenic markers *COL1* and osteocalcin (Figs 2B and 2C).

Hypoxia alone reduced gene expression of the chondrogenic marker *SOX9* at day 4 by 2-fold (p=0.006) compared to controls in hASCs (Fig 3), while the combination of hypothermia and hypoxia reduced *SOX9* gene expression at day 4 by 2.9-fold (p=0.006) compared to controls. As a result, the combination of hypothermia and hypoxia did not affect *SOX9* gene expression compared to hypoxic hASCs.

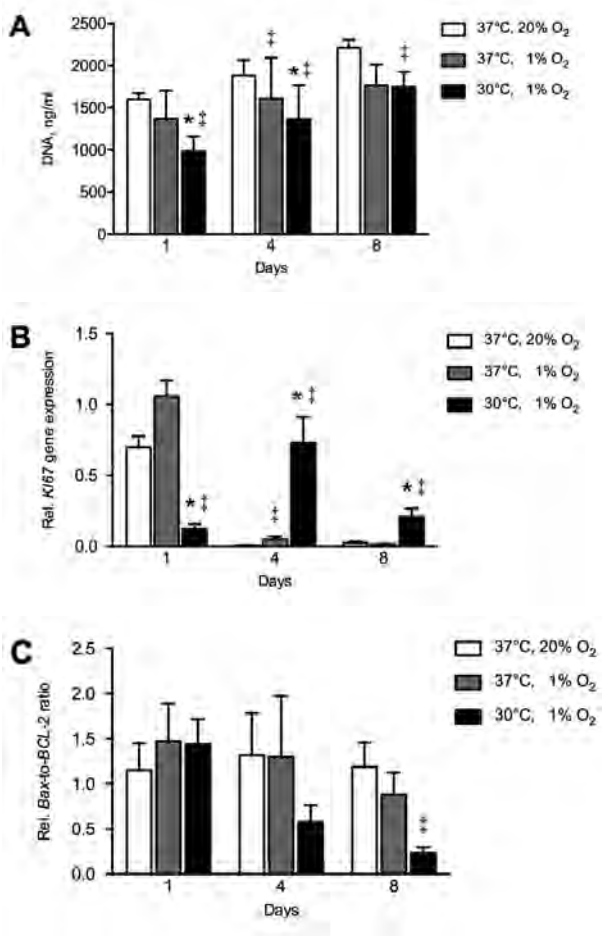
Hypoxia upregulated *VEGF-165* gene expression at day 1 by 3.5-fold (p=0.008), at day 4 by 15-fold (p=0.0005), and at day 8 by 2.5-fold (p=0.007), compared to controls (Fig 4A). The combination of hypothermia and hypoxia decreased *VEGF-165* gene expression at day 4 by 6-fold (p=0.007) and at day 8 by 2.1-fold (p=0.002), compared to hypoxia alone (Fig 4A). Hypoxia increased VEGF protein concentration at day 8 by 2.9-fold (p=0.003) compared to controls (Fig 4B). The combination hypothermia and hypoxia decreased VEGF protein concentration at day 8 by 1.9-fold (p=0.0011) compared to hypoxia (Fig 4B).

Hypoxia reduced NO production at day 1 by 1.3-fold (p=0.006), at day 4 by 1.9-fold (p=0.007), and at day 8 by 1.7-fold (p=0.011), compared to controls (Fig 5). The combination of hypothermia and hypoxia reduced NO production at day 1 by 1.4-fold (p=0.0009), at day 4 by 2.2-fold (p=0.017), and at day 8 by 2.1-fold (p=0.004), compared to controls. As a result,

NO production by hASCs under the combination of hypothermia and hypoxia was comparable to that by cells under hypoxia alone (Fig 5).

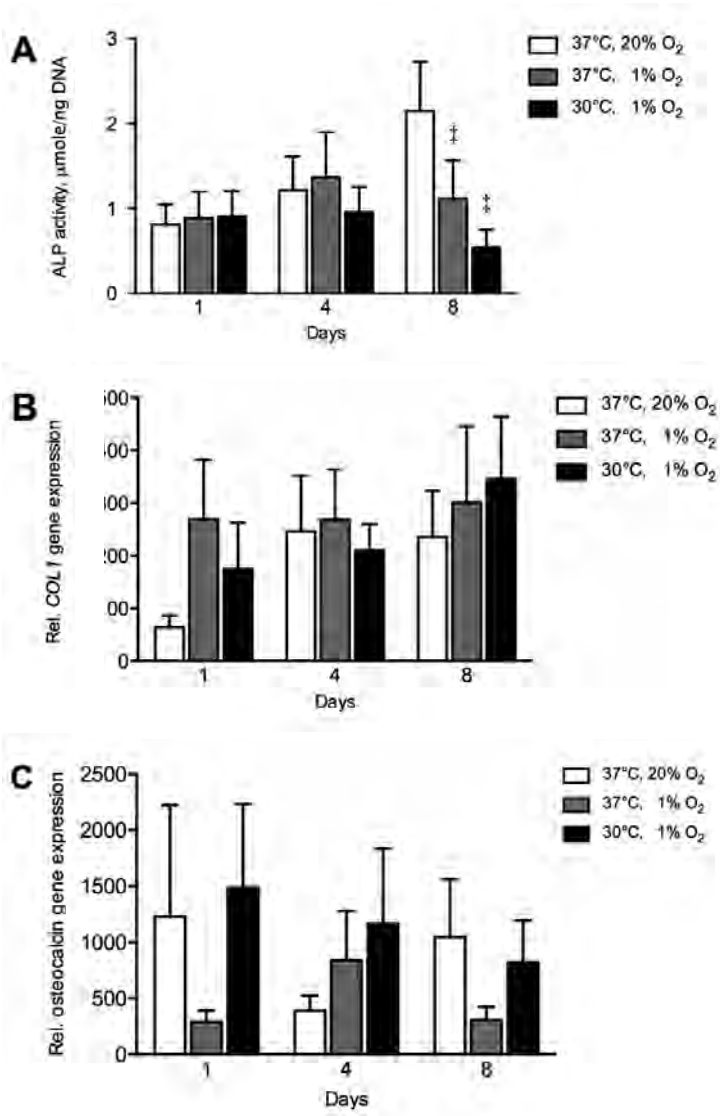
Hypoxia reduced total protein production rate at day 8 by 1.5-fold (p=0.002), compared to control hASCs (Fig 6). The combination of hypothermia and hypoxia similarly reduced total protein production rate at day 8 by 2.1-fold (p=0.001) compared to controls.

Figure 1. Effect of hypothermia and/or hypoxia on total DNA, *KI67* gene expression, and *BAX*-to-*BCL-2* gene expression ratio.



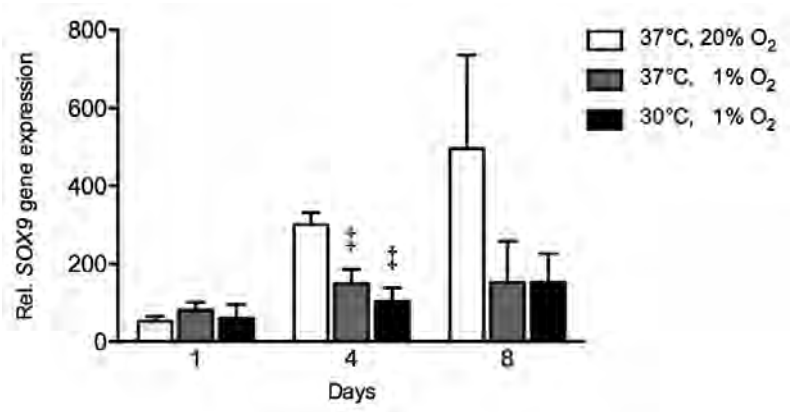
(**A**) Total DNA. The combination hypothermia and hypoxia decreased total DNA at day 1 by 1.4-fold and at day 4 by 1.2-fold, but not at day 8 under hypoxia. **(B)** *KI67* gene expression. Hypoxia upregulated *KI67* gene expression at day 4 by 5-fold compared to controls. The combination hypothermia and hypoxia downregulated *KI67* gene expression at day 1 by 8.8-fold, but increased *KI67* gene expression at day 4 by 15-fold, and at day 8 by 21-fold under hypoxia. **(C)** *BAX*-to-*BCL-2* gene expression ratio. The combination hypothermia and hypoxia did not affect *BAX*-to-*BCL-2* ratio under hypoxia. The combination hypothermia and hypoxia decreased *BAX*-to-*BCL-2* ratio at day 8 by 5.1-fold compared to controls. Values are mean ± SEM, n=10 from 3 independent experiments using ASCs obtained from 5 stem cell donors. *Significant effect of the combination hypothermia and hypoxia compared to hypoxia alone; ‡Significant effect compared to controls, p<0.05. Controls: 37°C, 20% O_2. Gene expression is expressed relative to the average expression of the three housekeeping genes.

Figure 2. Effect of hypothermia and/or hypoxia on ALP activity, *COL1*, and osteocalcin gene expression.



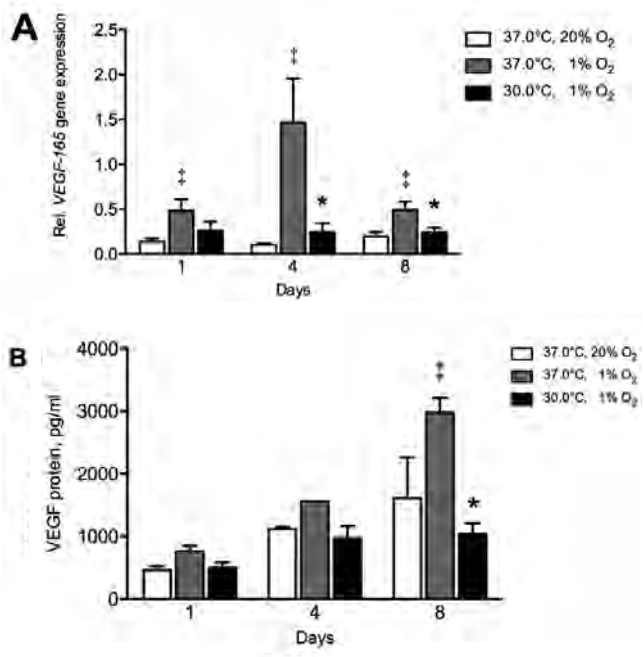
(A) ALP activity normalized for cell number. The combination hypothermia and hypoxia did not affect ALP activity under hypoxia. Hypoxia decreased ALP activity at day 8 by 4-fold compared to controls. **(B)** *COL1* gene expression. Hypoxia and the combination hypothermia and hypoxia did not affect *COL1* gene expression. **(C)** Osteocalcin gene expression. Hypoxia and the combination hypothermia and hypoxia did not affect osteocalcin gene expression. Values are mean ± SEM, from n=10 of 3 independent experiments using ASCs obtained from 5 stem cell donors *Significant effect of the combination hypothermia and hypoxia compared to hypoxia alone; ‡Significant effect compared to controls, $p<0.05$. Controls: 37°C, 20% O_2. Gene expression is expressed relative to the average expression of the three housekeeping genes.

Figure 3. Effect of hypothermia and/or hypoxia on *SOX9* gene expression.



The combination hypothermia and hypoxia did not affect *SOX9* gene expression under hypoxia. Hypoxia reduced *SOX9* gene expression at day 4 by 2.9-fold compared to controls. Values are mean ± SEM, from n=12 of 3 independent experiments using ASCs obtained from 6 stem cell donors. Gene expression is expressed relative to the average of the three housekeeping genes. ‡Significant effect compared to controls, $p<0.05$. Controls: 37°C, 20% O_2.

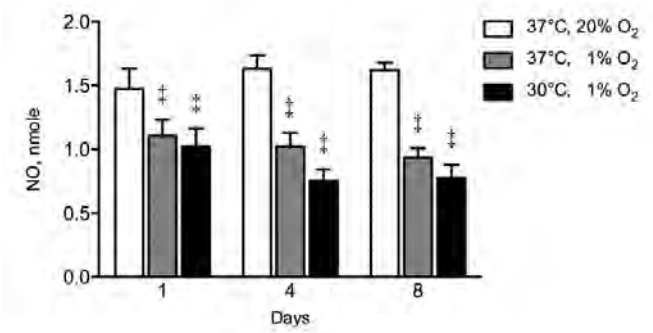
Figure 4. Effect of hypothermia and/or hypoxia on VEGF gene and protein expression.



(**A**) *VEGF-165* gene expression. The combination hypothermia and hypoxia decreased *VEGF-165* gene expression at day 4 by 6-fold and at day 8 by 2.1-fold under hypoxia. Hypoxia upregulated *VEGF-165* gene expression at day 1 by 3.5-fold, at day 4 by 15-fold, and at day 8 by 2.5-fold compared to controls. (**B**) VEGF protein expression. The combination hypothermia and hypoxia decreased VEGF protein concentration by 2.9-fold compared to hypoxia alone at day 8. Hypoxia increased VEGF protein concentration by 1.9-fold compared to controls at day 8. Values are mean ± SEM, from n=12 of 3 independent

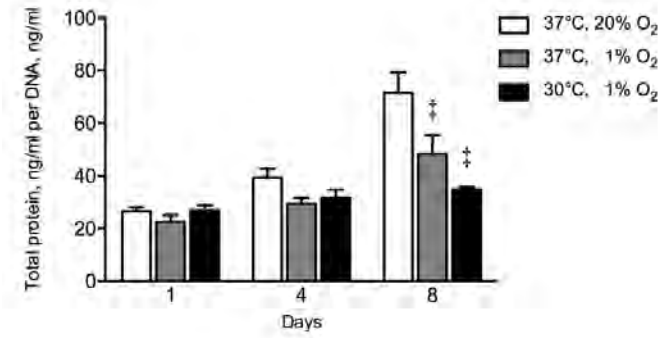
experiments using ASCs obtained from 6 stem cell donors for *VEGF-165* gene expression, and n=7 of 3 independent experiments from 4 stem cell donors for VEGF protein concentration. Gene expression is expressed relative to the average expression of the three housekeeping genes. *Significant effect of the combination hypothermia and hypoxia compared to hypoxia alone; ‡Significant effect compared to controls, $p<0.05$. Controls: 37°C, 20% O_2.

Figure 5. Effect of hypothermia and/or hypoxia on NO production.



The combination hypothermia and hypoxia did not affect NO production under hypoxia. Hypoxia decreased NO production at day 1 by 1.3-fold, at day 4 by 1.9-fold, and at day 8 by 1.7-fold compared to controls. Values are mean ± SEM, from n=12 of 3 independent experiments using ASCs obtained from 6 stem cell donors. ‡Significant effect compared to controls, $p<0.05$. Controls: 37°C, 20% O_2.

Figure 6. Effect of hypothermia and/or hypoxia on total protein content normalized for cell number.



The combination hypothermia and hypoxia did not affect total protein under hypoxia. Hypoxia decreased total protein at day 8 by 1.5-fold compared to controls. Values are mean ± SEM, from n=12 of 3 independent experiments using ASCs obtained from 6 stem cell donors. ‡Significant effect compared to controls, $p<0.05$. Controls: 37°C, 20% O_2.

Discussion

Hypothermia is used in fracture patients to enhance recovery [4]. It has been suggested that this enhanced recovery originates from modulation of the inflammatory reaction [2,3]. Hypothermia may also modulate the effects of ischaemia and hypoxic conditions that are prevalent in the wound environment, but whether the combined effect of hypothermia and hypoxia is beneficial for the cells responsible for callus formation is unknown.

The hypoxic environment in which osteoprogenitor cells naturally reside is thought to stimulate proliferation [29]. We found that in our culture conditions hypoxia actually inhibits MSC number. We hypothesized that hypothermia attenuates this hypoxia-induced inhibition of MSC number. We found that the combination of hypothermia and hypoxia reduced cell number and *KI67* gene expression compared to hypoxic hASCs already after 1 day of culture, but it stimulated *KI67* gene expression at day 4, probably leading to the observed catch-up effect in cell number after 8 days. This catch-up effect combined with unchanged apoptosis suggests increased proliferation of hASCs after hypothermia treatment of hypoxic MSCs. Hypothermia (35.5°C) has been reported to transiently reduce the number of osteoblasts cultured under normoxia [21]. It initially reduces cell number by 20% after 4 days of culture under normoxia and recovers to control levels after 7 days [21]. Under normoxia, hypothermia (33°C) was also reported to reduce DNA synthesis by 24% in bone marrow MSCs after 4 days of culture compared to normothermic MSCs [30]. We found that hypoxia reduced hASC proliferation at 37°C, which agrees with published data showing reduced proliferation of human bone marrow MSCs cultured under hypoxic conditions (1% O_2) [31]. Apparently, the effect of hypothermia on cell proliferation differs under hypoxic and normoxic conditions.

Hypothermia regulates the expression of various genes involved in necrotic and apoptotic pathways, such as the PI3K/Akt pathway, but the mechanisms have not been completely elucidated to date [32]. Hypothermia attenuates the increase in Bax gene expression associated with ischaemic brain damage in a rodent model [33]. Consequently, the pro-apoptotic effect of Bax protein on mitochondrial membrane potential is reduced by hypothermia, which may cause an anti-apoptotic effect [33]. We questioned whether apoptosis is stimulated by hypoxia, and whether hypothermia attenuates the stimulation of apoptosis in hypoxic MSCs. We found that apoptosis (assessed as *BAX*-to-*BCL-2* gene expression ratio) was not affected by hypoxia, nor did hypothermia modulate cell apoptosis under hypoxic conditions in hASCs.

We determined whether osteogenic differentiation is increased in hypoxia, and if

hypothermia attenuates the hypoxia-induced stimulation of hASCs. Diverging data exist about the effect of hypoxia on MSC differentiation [31,34–36]. Bone marrow-derived human MSCs cultured under a 2% oxygen tension for one month exhibit increased expression of early osteogenic differentiation markers osteonectin and alkaline phosphatase activity compared to cells grown under normoxic conditions [34]. A murine MSC line C3H/10T1/2 pre-incubated with the hypoxia mimicking agent $CoCl_2$ for 24 h shows enhanced osteogenic differentiation and mineralization [35]. In contrast, *in vitro* osteogenesis and chondrogensis is severely diminished in hASCs after 3 weeks of culture under a 2% oxygen tension [36]. A decrease in ALP activity in combination with increased expression of late markers for osteogenesis have been shown in hypoxic (1% O_2) human bone marrow stem cell cultures [31]. This suggests that a hypoxic environment might alter the timing of sequential gene expression in the osteogenic differentiation process, and consequently early osteogenic differentiation markers are reduced compared to late markers [31]. This is in partial accordance with our results, as we showed that hypoxia significantly reduced cell-associated ALP activity, but did not affect *COL1* and osteocalcin compared to control hASCs. It is likely that the timing and degree of hypoxia influences MSC differentiation. Enhanced expression of late differentiation markers might be possible at later time points than day 8.

Under normoxia, hypothermia (35.5°C) has been shown to reduce ALP activity, osteocalcin, and Col1 gene expression, and to decrease bone formation by 70% in murine osteoblasts [21]. The combination of hypothermia and hypoxia in our experiments might thus be expected to result in a stronger inhibition of MSC differentiation than hypoxia alone. In our study with hASCs we found that, under hypoxia, hypothermia did not affect cell-associated ALP activity, *COL1* gene expression, nor osteocalcin expression in hASCs.

We found that hypoxia reduced gene expression of the chondrogenic marker *SOX9* after 4 days of culture compared to controls. *SOX9* gene expression is significantly upregulated in human bone marrow stem cells cultured under 1% O_2 compared to normoxia after 14 days [31]. Moreover, human embryonic stem cells cultured under hypoxia (5% O_2) show increased *SOX9* gene expression after 14 days [37]. Murine C3H10/T1/2 cells pre-incubated with a hypoxia mimicking agent, show increased Sox9 gene expression after 3 days of culture [35]. Hypoxia (3% O_2) has also been shown to enhance chondrogenesis in ovine bone marrow MSCs cultured on porous scaffolds for 14 days compared to normoxia [29]. Please note that under normal physiological conditions, human bone marrow cells reside under 6% O_2 [38]. When cells reside under 3-5% O_2 this might not be considered as a severe hypoxic condition (as in our study) [38]. This could explain the difference between our results and the results

reported in literature. In addition, our 2-dimensional culture conditions do not naturally favour chondrogenic differentiation.

We expected an upregulation of *SOX9* gene expression under hypoxia since chondrogenesis is driven by HIF-1α [39], and HIF-1α is upregulated under hypoxia [1]. HIF-1α also affects VEGF [12,39] and we showed a significant upregulation of *VEGF-165* gene expression under hypoxia after 4 days of culture, even though we were unable to demonstrate an effect of hypoxia on *SOX9* gene expression. Hypothermia significantly reduced *VEGF-165* gene expression, as well as VEGF protein expression under hypoxia, yet we were unable to demonstrate an effect of hypothermia on *SOX9* gene expression under hypoxia in hASCs. Further analysis on HIF-1α might elucidate these intriguing results.

We found that *VEGF-165* gene expression and VEGF protein expression were reduced by hypothermia under hypoxia. This is in accordance with another study showing that VEGF production decreases by 30% in hypoxic (1% O_2) retinal pigment epithelial (ARPE-19) cells exposed to moderate (34°C) hypothermia [40]. VEGF is a bone-metabolism cytokine that stimulates the proliferation and chemotactic migration of osteoblast precursor cells [11]. Thus reduced VEGF levels may decrease osteoblast proliferation and possibly also differentiation, yet we did not find a reduction in ALP activity or osteocalcin gene expression as a result of hypothermia in our hypoxic culture conditions. This suggests that the early stages of fracture healing are not adversely affected by hypothermia under hypoxic conditions. Being a powerful inducer of angiogenesis, VEGF is important for the later stages of bone healing when vascularization of the callus is warranted [41]. Hence reduced VEGF levels as a result of hypothermia might have implications for the later stages of bone healing.

Since hypoxia increases oxidative stress, we hypothesized that hypoxia stimulates NO production, and that hypothermia attenuates the hypoxia-induced increase in NO production of MSCs. NO production was consistently reduced under hypoxia compared to control hASCs. Hypothermia did not affect NO production by hASCs under hypoxia. In physiological conditions, a low NO level maintains vasculature tone [42]. In pathological conditions, such as hypoxia in ischaemic-reperfusion injury, NO levels will ultimately increase by the activity of inducible NO synthase (iNOS) after reperfusion, thereby contributing to overall oxidative stress [43,44]. NO production requires oxygen since it is synthesized from L-arginine and O_2. In our experiments, there is no increase in oxygen tension as occurs during reperfusion after ischaemia. This might explain the lack of an increase in NO production by hASCs cultured under hypothermia. Mast cells likely play a key role in iNOS-mediated augmentation of

oxidative stress, and lack of these cells might not lead to a full-blown cascade with increased NO concentrations [44].

Some care must be taken when interpreting our results. We used hASCs since these cells can differentiate along the osteogenic lineage, they can be stimulated by hypoxic conditions and are readily available, and thereby resemble periosteum-derived osteoprogenitor cells responsible for bone repair *in vivo* [8,45–47]. The frequency of BMSCs in human bone marrow is low, and proliferative and differentiation capacity of BMSC is partially lost during cell expansion [48]. However, in contrast to bone marrow, adipose tissue contains a high stem cell to volume ratio [49], and it can be processed within a short time frame to obtain highly enriched ASC preparations. ASCs show many similarities with BMSCs with regard to surface marker profiles, multi-lineage potential, and growth properties [50,51]. The hASCs used in our study have been characterized previously by our group [22,23,51]. Our *in vitro* data provide insight in the mechanism of the effect of cryotherapy on MSCs, but *in vivo* experiments are needed to draw firm conclusions about the effect of cryotherapy on bone repair. Note that we tested the effect of hypothermia in cells under hypoxia, but not normoxia, since this was beyond the scope of our study, where we aimed to mimic the hypoxic fracture environment. Cell culture was performed under ambient oxygen levels, and therefore the cells were exposed to acute hypoxia during the course of the experiments. The naturally occurring stem cell niche in which most stem cells grow or reside is a hypoxic environment, and therefore our control condition is in fact hyperoxic and might alter hASC characteristics [6]. Most studies cited [3,18,19,21,30,33] address hypothermia at near physiological temperature (33-37°C). However cryotherapy causes hypothermia at a lower temperature, i.e. outside the physiological range (<33°C). Cryotherapy lowers the temperature to 23°C in healthy individuals at 1.5 cm below the subcutaneous fat layer [15]. Currently no reports are available that show temperature decline at the bony level after induced hypothermia treatment in fracture patients. One study measuring the temperature decline found that after induced hypothermia at 1 cm (23.5°C) and 2 cm (26.4°C) below the subcutaneous fat layer found that temperature decline during induced hypothermia and tissue depth are inversely related [52]. Based on an estimated distance between the subcutaneous fat layer and the bone (~3 cm), and the inverse relationship between temperature decline by hypothermia and tissue depth we estimated the temperature at a deeper bony level of the thigh to be 30°C.. We applied intra-hypoxic hypothermia, i.e. hypothermia commenced and continued at the same time and as long as hypoxia treatment. Hence care should be taken when translating these *in vitro* results

to an *in vivo* situation, since clinically hypoxia or ischaemia is usually present before starting hypothermia treatment, and hypothermia treatment might not be applied continuously.

In conclusion, our data show that under hypoxia, hypothermia reduced *VEGF-165* gene expression and VEGF protein expression, but did not affect cell number, nor osteogenic or chondrogenic differentiation, or NO production by hASCs. Decreased VEGF gene and protein expression might ultimately reduce vasculogenesis, which may impair later stages of bone healing *in vivo*.

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Chapter 8

Summary and general discussion

The present thesis addresses several aspects regarding continuous-flow cryocompression therapy (CFCT) after hip fracture surgery in order to treat pain and mitigate postoperative haemorrhage. By exploring its efficacy and by determining the thermodynamic aspects of CFCT an attempt is made to elucidate the treatment mechanism and define deep tissue temperature reduction. Furthermore, by use of a mesenchymal stem cell (MSC) model, we aimed to provide insight in the mechanism of effect of cryotherapy on MSC's. Thorough knowledge of these aspects will help clinicians to decide whether to instigate this therapy for patients after hip fracture surgery.

Cryotherapy techniques, indications and outcomes

The traditional application of cryotherapy concerns applying a frozen substance or ice water to the target area, but nowadays a wide array of commercially available machines applies the cooling principles of cryotherapy. Although each apparatus' design is unique, a certain distinction can be made. While the first machines applied cryotherapy intermittently (manual 're-chilling' of the wrap was necessarily), the current machines are equipped with an internal pump facilitating an adjustable continuous-flow of ice-cold water, and most are also embedded with a dynamic pneumatic pressure adjunct. The pneumatic pressure adjunct increases the density of the target area, thereby further augmenting the cooling efficacy[1]. In **chapter 2** we provided an overview of fluid-based continuous-flow cryotherapy with and without a compression adjunct that is applied in the acute recovery phase of surgical repair for musculoskeletal injury of the lower extremity.

Cryotherapy is applied after total knee arthroplasty (TKA)[2,3], unicondylar knee arthroplasty[4], anterior cruciate ligament reconstruction[5–10], footfractures[11], arthroscopy[12] and after total hip arthroplasty (THA)[13–15]. In our overview half of the caseload in which cryotherapy is applied is after knee surgery. Not surprisingly in TKA and unicondylar knee arthroplasty high perceived postoperative pain scores that may limit postoperative mobilisation are not uncommon[16,17]. Cryotherapy is frequently instigated as part of a multimodal analgesic regimen, but only one study in our overview reported a profound analgesic effect after TKA[13], and the remainder of the studies found no convincing clinical effect on pain or postoperative haemorrhage. The moderate effect of cryotherapy is confirmed in meta-analyses[2,3], and the compression adjunct does seem to increase treatment efficacy after TKA. In contrast to knee surgery, few reports exist that assess the efficacy of cryotherapy after total hip arthroplasty. We only found three studies that reported on the analgesic efficacy of cryotherapy after elective total hip arthroplasty for end-stage

osteoarthritis (THA-OA). Herein two studies report an interesting decline in early pain scores as well as a moderate decline in analgesic use, but no clinical relevant results implicate that postoperative haemorrhage is mitigated. Strikingly, only one study assessed cryotherapy after acute bony trauma[11]. Stöckle et al. found no analgesic efficacy of cryotherapy, but did demonstrate a reduction of swelling after foot fracture surgery. It is remarkable that CFCT is not instigated more often for analgesic purposes after a sustained or fixated fracture, since fractures are generally accompanied by soft tissue trauma and oedema that are aggravated by subsequent surgical fixation. Due to this duplicate trauma these patients, and especially patients with extensive soft tissue trauma such as in hip surgery, should benefit most from CFCT.

Cryotherapy can be judged as safe, only 1.51% (9 of 596 patients) that received cryotherapy reported mild adverse events, which resolved after cessation. In addition incidence of serious cryotherapy-related complications is reported to be 0.0023%[18].

This study provides a clear overview that reveals significant treatment heterogeneity, and remarkably found only one study that assessed cryotherapy in fractures. All studies applied cryotherapy in a different way: duration, frequency, type of machine, use of a compression adjunct (static or dynamic), and temperature setting. These inconsistencies illustrate the lack of an evidence-based derivative that guides optimal treatment. Current recommendations are mostly based on old data and expert opinions that are based on clinical outcomes[13]. Since each treatment locus on the body has unique characteristics this derivative may vary. Future research should focus on a better understanding of the physiological effects of cooling; consequently a clear derivative should be developed that advocates how cryotherapy treatment can be optimized.

Cryotherapy after hip surgery

In **chapter 3**, we aimed to determine the efficacy of CFCT after elective THA-OA. We demonstrated CFCT to reduce postoperative haemorrhage at day one, and subjects were positive about CFCT, but no effects were observed on postoperative pain or analgesic use. Shortly after this study had finished fast track protocols for THA-OA were implemented in our hospital that employ aggressive analgesic strategies together with immediate and stringent postoperative mobilisation. Applying cryotherapy parallel to these fast track protocols does not seem feasible *in a clinical setting*. However due to the positive findings in our THA-OA study, the relative similarity between THA-OA and hip fracture patients and that hip fracture

patients experience severe postoperative pain with duplicate trauma[16,19], we conducted a more extensive variant of this study in a multicentre setting in a hip fracture population.

Hip fracture patients are a heterogeneous group treated surgically with cannulated hip screws, (hemi) arthroplasty, dynamic hip screw or with a intramedullary hip nail according to fracture type and the clinical patient profile[20,21]. In hip fracture patients altered pharmacodynamics and kinetics narrow the therapeutic window and consequently limit the use op opioid-based analgesics, combined with the painful nature of a hip fracture this leads to increased difficulty in providing adequate analgesia to hip fracture patients[16,19]. In **chapter 4 and 5**, we respectively report the design and the results of our multicentre trial that aimed to determine the efficacy of CFCT in the postoperative recovery phase of hip fracture patients. We found a mild reduction in numeric rating scale pain after 3 days of complete (per protocol) treatment, but no differences were found in analgesic use or on the other outcome parameters. The reduction in pain was not demonstrated in the regular intention to treat analysis, where 28% reported discomfort (usually cold intolerance) and 15.6% dropped out prematurely (usually at the first treatment). The remainder of the subjects were generally positive about their experiences with CFCT. The complication rates between groups were not significantly different.

The study in **chapter** 5 is the first to provide the results of CFCT, a non-pharmacological analgesic intervention, in a frail elderly hip fracture population. Conducting research with elderly remains challenging as declined perception and comprehension to understand study specific actions may cause additional anxiety and stressors, both of which have been related to an increase pain perception, which consequently may obfuscate a treatment effect[22]. It may also explain the high drop out rates that was observed in our study, as failure to comprehend the intention of study interventions will inevitably lead to uncooperative or unwilling subjects. Still, we were unable to demonstrate neither a significant decline in analgesic use nor a decline in postoperative haemorrhage. The staggering 50% decline in analgesic use that was previously reported seems unrealistic[13], as the other sparse studies report diverging analgesic efficacy[14,15].

In sub analyses we did not find evidence that the type of surgery was related to CFCT efficacy. Postoperative pain originates from soft tissue trauma and from bone trauma, in case of minimally invasive intramedullary hip nail, dynamic hip screw or cannulated hip screws the fracture site allows for painful micro motion[19], while in (hemi) arthroplasty the fracture site is removed, but at the expense of increased soft tissue trauma. Therefore dynamic (on locomotion) pain scores are much lower after (hemi) arthroplasty and fixation of undisplaced

hip fractures[19]. We hypothesized that CFCT only reduces pain that originates from soft tissue trauma, and does not reduce the pain that originates from deeper fracture micro motion pain. Hence static pain was measured as opposed to dynamic pain that incorporates fracture micro motion to a degree. Bone injury and micro motion are more painful than soft tissue injury because the periosteum has the lowest pain threshold of the deep somatic tissue. Currently it is unknown if CFCT penetrates to the bone level where it might reduce bone-derived pain, therefore future trials should assess pain during specific functions e.g. walking to assess for a analgesic effect on bone-derived pain.

Continuous-flow cryocompression therapy thermodynamics

The analgesic efficacy of cryotherapy differs in various treatment foci such as in THA, TKA and after fixation or (hemi) arthroplasty of hip fractures, and it is not clearly established how cryotherapy exerts its analgesic effect. Two theories can be proposed: either superficial via an interaction on nerve conduction, or deep via an interaction with tissue metabolism and immunomodulation, or a combination of these theories. The demonstrated moderate efficacy of cryotherapy in TKA and the diverging results in the sparse THA reports are puzzling, but might be explained by the varying extent of soft tissue trauma. A major difference between the knee and hip joint is the extent of the connective tissue layer. While the knee virtually lacks a fat layer, a layer of up to several centimetres thick surrounds most hip joints. This connective (and fat) tissue layer has significant implications when it comes to the cooling efficacy of cryotherapy because penetration depth of cryotherapy and thickness of this subcutaneous tissue layer are strongly inversely related[23]. This inverse relation may contribute to the observed lack of efficacy observed in THA patients.

In **chapter 6**, we aimed to define deep tissue temperature during CFCT in postoperative hip fracture patients, by using measured skin temperature as input parameter for a simple numerical model. Second, the association between tissue temperature distribution and pain reduction was investigated to assess cryotherapy-induced analgesia of soft tissue derived pain. Thereby trying to substantiate our hypothesis stating that: CFCT does not reach the bone level, but that increased soft tissue penetration is associated with reduced numeric rating scale pain levels, consequently providing evidence that CFCT analgesia is mediated by attenuating soft tissue derived pain. We found CFCT to reduce temperature up to 3 cm in postoperative hip fracture patients. Forty-two per cent of our patients had a soft tissue layer of less than 3 cm in our study group, thus in these cachectic patients CFCT reduces temperature at the bone, where it might have implications for bone tissue healing when treated for a prolonged period

of time. However no association between tissue temperature distribution and pain reduction was demonstrated. Pain in hip fracture patients originates from traumatized skin, as well as trauma to muscle and bone tissue. The cryotherapy-induced skin analgesia apparently is insufficient for patients to perceive, because an equivalent or greater amount of pain originates from the deeper muscular and/or bone regions. The lack of an association between tissue temperature distribution and pain reduction illustrates this hypothesis, stating that CFCT only provides skin analgesia, and insufficiently or incompletely cools muscle and bone tissue in order to provide analgesia at these regions.

Effects of cryotherapy in a mesenchymal stem cell model

Cryotherapy is used in an attempt to reduce pain in various musculoskeletal injuries, but its effect on the cells responsible for bone healing is unknown. It is well known that cryotherapy reduces tissue metabolism[24], but in the case of a recent fracture or fixation of a fracture, an elevated metabolic state, able to produce fracture callus and bone remodelling is warranted. Since we demonstrated that CFCT is able to reduce temperature at the bone level in cachectic patients in **chapter 6**, it should be explored if cryotherapy adversely affects osteoblast precursor proliferation and differentiation, cell functions that are responsible for bone tissue repair, in order to avert iatrogenic non or delayed unions of fractures.

Chapter 7 presents the results of hypothermia in a MSC model under hypoxic conditions, thereby providing insight in the mechanism of the effect of cryotherapy on MSCs. In these experiments hypoxia was used as a surrogate for a sustained fracture. The combination of hypothermia and hypoxia decreased *VEGF*-165, which is a marker for vasculogenesis. Although differentiation and proliferation of MSC's were uninfluenced, the blunting of *VEGF*-165 could have implications for callus vascularisation, a later stage of bone healing. Our *in vitro* results implicate that hypothermia treatment *in vivo* that is applied to alleviate pain and inflammation, is not likely to harm early stages of callus formation but might have implications for later stages.

Our study is the first to describe the *in vitro* effects of hypothermia in a MSC model under hypoxia. Although more *in vivo* research is necessarily in order to draw firm conclusions about the effect of cryotherapy on bone repair. Our results from **chapter 6** demonstrated that in cachectic patients CFCT reduces temperature in a degree equivalent to our MSC experiments. Therefore application of CFCT might have adverse effects on the later stages of bone healing if it is applied for a prolonged period of time.

Conclusions

The studies presented in this thesis have led to the following conclusions regarding the application of CFCT after hip fracture surgery and hypothermia:

- Cryotherapy is predominately applied after (semi) elective surgery, where its application for the various musculoskeletal injuries is heterogeneous and safe, it offers a mild reduction of opioid consumption and blood loss;
- Continuous-flow cryotherapy reduces postoperative blood loss after THA-OA one day after surgery and is valued by patients, it does not reduce pain;
- Continuous-flow cryotherapy has no analgesic benefits in the acute postoperative recovery phase of hip fracture surgery, nor does it reduce postoperative blood loss;
- In patients with soft tissue skin layer of less than 3 cm continuous-flow cryocompression therapy penetrates to the bone level in hip fracture patients;
- Tissue temperature reduction by continuous-flow cryocompression therapy and pain perception of hip fracture patients is not related. This might suggest that cryotherapy-induced analgesia originates from skin analgesia, rather than analgesia of muscle or bone derived pain;
- Hypothermia decreases *VEGF-165* gene and protein expression, but does not affect differentiation, or apoptosis of MSCs cultured under hypoxia. This implicates that hypothermia treatment *in vivo*, applied to alleviate pain and inflammation, is not likely to harm early stages of callus formation.

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Chapter 9

Nederlandse samenvatting

In dit proefschrift worden verschillende aspecten belicht van continue-stroming cryocompressietherapie (CSCT). Deze therapie wordt toegepast na heupfractuurchirurgie met de intentie pijn te verlichten en postoperatief bloedverlies te verminderen. Door de werkzaamheid en de thermodynamische eigenschappen van CSCT te bepalen wordt gepoogd de manier waarop CSCT werkt te verklaren. Daarnaast wordt middels een mesenchymaal stamcel (MSC) model inzicht gegeven over de totstandkoming van het waargenomen effect van cryotherapie op deze MSC's. Grondige kennis van deze aspecten kunnen clinici ondersteunen in de besluitvorming deze therapie te gebruiken voor heupfractuurpatiënten die herstellen na een operatie.

Cryotherapie technieken, indicaties en uitkomsten

Traditioneel wordt bij cryotherapie gebruikgemaakt van een bevroren substraat of ijswater, en tegenwoordig bestaat er een scala aan commercieel beschikbare machines die deze therapie toepassen. Hoewel elk apparaat hetzelfde principe van cryotherapie toepast, zijn er duidelijke verschillen. De eerste ontwerpen pasten cryotherapie intermitterend toe (de koelwrap diende handmatig opnieuw gekoeld te worden), terwijl de hedendaagse varianten zijn uitgerust met een interne pomp waarmee de continue-stroom van ijswater kan worden gereguleerd en waarmee tevens dynamisch pneumatische druk kan worden gegenereerd. De pneumatische druk zorgt ervoor dat het doelgebied wordt gecomprimeerd en daarmee de werkzaamheid verbeterd[1]. In **hoofdstuk 2** geven we een overzicht van de effectiviteit van op vloeistof gebaseerde, continue-stroming cryotherapie met én zonder pneumatische druk in de acute herstelfase van de chirurgische behandeling van musculoskeletale letsels van de onderste extremiteit.

Uit dit overzicht is op te maken dat cryotherapie wordt toegepast na totale knie arthroplastiek (TKA)[2,3], unicondylaire knie arthroplastiek[4], voorste kruisband reconstructie[5-10], voetfracturen[11], arthroscopie[12] en totale heup arthroplastiek (THA)[13-15]. De helft van toepassingen van cryotherapie als pijnstiller betrof kniechirurgie. In de lijn der verwachting worden in de herstelfase van TKA en unicondylaire knie arthroplastiek hoge postoperatieve pijnscores gerapporteerd, die postoperatieve mobilisatie kunnen vertragen. Hoewel cryotherapie vaak deel uitmaakt van een multimodale pijnbestrijdingstrategie, blijkt dat slechts één onderzoek uit ons overzicht een duidelijk pijnstillend effect na TKA vond[13], en de overige studies geen klinisch effect op pijn of postoperatief bloedverlies vonden. De minimale effectiviteit van cryotherapie na TKA wordt bevestigd in meta-analyses[2,3], maar de pneumatische druk lijkt de effectiviteit iets te verbeteren. In tegenstelling tot kniechirurgie

zijn er na electieve THA vanwege eindstadium osteoarthrose (THA-OA) slechts enkele studies die het pijnstillend effect van cryotherapie onderzoeken. In twee van deze studies wordt er een interessante afname in vroege pijnscores gevonden en tevens ook enige afname van analgeticagebruik. Er zijn echter geen aanwijzingen dat postoperatief bloedverlies wordt verminderd door cryotherapie. Opmerkelijk genoeg is er slechts één studie die de werkzaamheid van cryotherapie na acuut bottrauma heeft onderzocht[11]. Stöckle et al. vonden geen pijnstillende werking van cryotherapie, maar demonstreerden wel een afname van de postoperatieve zwelling na voetfractuurchirurgie. Het is opmerkelijk dat CSCT niet vaker wordt ingezet bij fracturen, aangezien fracturen meestal gepaard gaan met wekedelentrauma en oedeem die beide verergerd worden door een noodzakelijke chirurgische fixatie. Door dit tweevoudige trauma zullen deze patiënten, en in het bijzonder patiënten met uitgebreid wekedelentrauma - zoals bij heupchirurgie - het meest profiteren van CSCT.

Cryotherapie wordt beschouwd als veilig. Slechts 1,51% van de patiënten (9 op de 596) die met cryotherapie werden behandeld rapporteerde milde bijwerkingen, welke verdwenen bij het staken. Daarnaast is de incidentie van ernstige cryotherapie-gerelateerde complicaties met ongeveer 0,0023% laag[18].

De studie in **hoofdstuk 2** geeft een duidelijk overzicht waaruit valt te concluderen dat er behoorlijke behandelheterogeniteit bestaat en er slechts één studie is verricht naar de werkzaamheid bij fracturen. Alle studies pasten cryotherapie toe op een andere manier; duur, frequentie, type apparaat, gebruik van een pneumatische druk (statisch of dynamisch) component en temperatuurinstelling. Deze variatie illustreert een gebrek aan een *evidence-based* behandelalgoritme. Huidige aanbevelingen zijn veelal gebaseerd op oude data en *expert opinions,* die vervolgens gebaseerd zijn op klinische uitkomsten[13]. Aangezien elk doelgebied op het lichaam uniek is, zal dit behandelalgoritme variëren per doelgebied. Toekomstig onderzoek moet zich richten op een beter begrip van de fysiologische effecten van koeling. Aansluitend zou een algoritme moeten worden geformuleerd dat de werkzaamheid van deze therapie optimaliseert.

9

Cryotherapie na heupchirurgie

In **hoofdstuk 3** hebben we de werkzaamheid van CSCT na THA-OA onderzocht. We hebben aangetoond dat CSCT postoperatief bloedverlies verminderd op dag 1 en dat deelnemers positief zijn over hun ervaringen met CSCT. Desondanks werden er geen effecten gevonden op postoperatieve pijn of gebruik van pijnstillende medicatie. Vlak na de afronding van deze studie werden er *fast-track* protocollen ingevoerd bij deze categorie patiënten in ons

ziekenhuis. In deze protocollen wordt er gestreefd naar een snelle en intensieve postoperatieve mobilisatie. Het in onderzoeksverband vaststellen van de werkzaamheid van cryotherapie bij deze categorie patiënten, parallel aan deze protocollen, lijkt niet haalbaar in een klinische setting. Gezien de relatieve gelijkenis tussen THA-OA en heupfractuurpatiënten, de hevige postoperatieve pijn die heupfractuurpatiënten ervaren[16,19], en de positieve bevindingen in onze THA-OA studie hebben we een grotere variant van deze studie uitgevoerd bij een heupfractuurpopulatie.

Heupfractuurpatiënten zijn een heterogene groep die nagenoeg altijd chirurgische behandeling behoeft in de vorm van gecanuleerde schroeven, dynamische heupschroef of met een intramedullaire heupnagel, afhankelijk van het fractuurtype en het patiëntenprofiel[20,21]. Bij heupfractuurpatiënten is de farmacodynamiek en -kinetiek door hoge leeftijd en comorbiditeit veranderd, wat tot een verkleind therapeutisch raamwerk leidt en waardoor er minder ruimte is voor het gebruik van opioïde-gebaseerde pijnstilling. De combinatie van een verkleind raamwerk en de pijnlijke aard van een heupfractuur maakt het verzorgen van goede pijnstilling voor deze categorie patiënten uitdagend[16,19]. In **hoofdstuk 4 en 5**, worden respectievelijk het ontwerp en de resultaten van onze multicenter studie besproken, waarin we de werkzaamheid van CSCT in de postoperatieve herstelfase van heupfractuurpatiënten onderzochten. We toonden een milde afname aan in pijnbeleving na drie dagen complete (volgens protocol) afgeronde behandelingen, maar er werden geen verschillen gevonden in het gebruik van pijnstillende medicatie of bij de andere uitkomsten. De afname in pijn werd echter niet gevonden in de *intention-to-treat* analyse, waar 28% ongemak rapporteerde (overwegend koude intolerantie) en 15,6% voortijdig uitviel (meestal bij de eerste behandeling). De overige deelnemers waren over het algemeen positief over hun ervaringen met CSCT. Er werden geen verschillen gezien in complicaties tussen de groepen.

In **hoofdstuk 5** wordt voor het eerst de werkzaamheid van de niet-farmacologische pijnstillende interventie CSCT beschreven in een kwetsbare oudere heupfractuurpopulatie. Het doen van onderzoek met kwetsbare ouderen is uitdagend gezien afgenomen begrip en onvermogen om specifieke studiehandelingen te begrijpen extra stressoren en onrust met zich mee kunnen brengen, die beide weer gerelateerd zijn aan toegenomen pijnperceptie. Dit kan een behandeleffect maskeren[22]. Deze stressoren kunnen ook de hoge uitval in onze studie verklaren, aangezien onbegrip jegens studiespecifieke handelingen zeer waarschijnlijk leidt tot niet-meewerkende deelnemers. We hebben geen significante afname in het gebruik van pijnstillende medicatie of postoperatief bloedverlies kunnen aantonen. De indrukwekkende 50% afname in het gebruik van pijnstillers door CSCT die eerder genoemd werd lijkt

onrealistisch[13], mede gezien het feit dat de overige studies uiteenlopende resultaten rapporteren over de werkzaamheid[14,15].

In subanalyses werd geen relatie gevonden tussen het type operatie en effectiviteit van CSCT. Postoperatieve pijn wordt veroorzaakt door zowel wekedelen- als bottrauma. Bij de minimaal invasieve operatietechnieken van de intramedullaire heupnagel, dynamische heupschroef en de gecanuleerde schroeven staat de gestabiliseerde fractuur nog pijnlijke microbeweging toe[19]. Terwijl bij (hemi-)arthroplastiek de fractuur verwijderd wordt ten koste van een groter dissectievlak c.q. wekedelentrauma. Om die reden zijn dynamische pijnscores (bij beweging) lager na (hemi-)arthroplastiek en fixatie van niet-verplaatste heupfracturen waar fractuurbeweging niet aanwezig is[19]. We hypothetiseren dat CSCT alleen pijn verminderd die veroorzaakt wordt door het wekedelentrauma, en dat het de diepere, door bot veroorzaakte pijn ongemoeid laat. Daarom werd statische pijn (in rust) gemeten in tegenstelling tot dynamische pijn, dat ook een deel van de pijn vanuit fractuur microbeweging incorporeert. Pijn door bottrauma en gerelateerde microbeweging zijn pijnlijker dan wekedelentrauma omdat het periost de laagste pijndrempel heeft van de somatische weefsels. Op dit moment is vanuit de huidig beschikbare onderzoeken en literatuur niet duidelijk of koeling van CSCT penetreert tot botniveau, waar het botgerelateerde pijn verminderd. Om die reden zijn toekomstige studies nodig die pijn onderzoeken tijdens specifieke handelingen, zoals lopen, om zo een klinisch pijnstillend effect op botgerelateerde pijn vast te stellen.

Continue-stroming cryocompressietherapie thermodynamica

Het pijnstillende effect van cryotherapie loopt uiteen bij verschillende behandelindicaties zoals THA, TKA en na fixatie of hemiarthroplastiek van heupfracturen. Verder is het ook niet duidelijk hoe cryotherapie zijn pijnstillende effect sorteert. Twee theorieën bestaan: een oppervlakkige route waarin cryotherapie een interactie heeft met zenuwgeleiding, of een diepe route waar het interfereert met weefselmetabolisme en immunomodulatie, of een combinatie van deze theorieën. De minimale werkzaamheid die gevonden wordt bij TKA en de uiteenlopende resultaten die gevonden worden in de weinige THA studies zijn raadselachtig, maar kunnen mogelijk worden verklaard door een variatie in de omvang van het wekedelentrauma. Een groot verschil tussen de knie en de heup is de omvang van een bindweefsellaag. Bij de knie ontbreekt er praktisch een isolerende bindweefsellaag, terwijl deze laag in de heup enkele centimeters dik kan zijn. Deze bind- en vetweefsellaag speelt een belangrijke rol bij het koelen van weefsel want bestaat een omgekeerde relatie tussen de

penetratie van cryotherapie en de dikte van deze onderhuidse bindweefsellaag[23]. Deze omgekeerde relatie kan het ontbreken van een effect in THA verklaren.

In **hoofdstuk 6**, hebben we met simpel numeriek model temperatuur in de diepere weefsels getracht te voorspellen tijdens CSCT in postoperatieve heupfractuurpatiënten, waarbij huidtemperatuur als parameter werd gebruikt. Daarnaast hebben we een associatie tussen de verdeling van weefseltemperatuur en pijnafname onderzocht om op deze manier een cryotherapie-geïnduceerde pijnverlichting van het wekedelentrauma vast te stellen. Hiermee trachten we onze hypothese te onderschrijven welke stelt dat: CSCT het botniveau niet bereikt, maar dat een toegenomen penetratie van weke delen is geassocieerd met een afname in pijnbeleving. Deze associatie zou kunnen ondersteunen dat klinisch waargenomen pijnverlichting door CSCT voortkomt uit een pijnreductie van het wekedelentrauma. We hebben gevonden dat CSCT tot 3 cm diepte de temperatuur verlaagd in heupfractuurpatiënten. Tweeënveertig procent van onze patiënten had een bindweefsellaagdikte van minder dan 3 cm, dus bij deze cachectische patiënten verlaagt CSCT de temperatuur op het botniveau. Dit kan implicaties hebben voor botgenezing indien de therapie voor langere tijd wordt toegepast en de relatieve hypothermie hiermee voortduurt. Echter, we hebben geen associatie gevonden tussen weefseltemperatuurverdeling en pijnreductie. Pijn in heupfractuurpatiënten wordt niet uitsluitend veroorzaakt door het wekedelentrauma, maar ook vanuit dieper spier- en bottrauma. Het pijnstillende effect van cryotherapie op de huid is kennelijk van onvoldoende omvang om voor patiënten opgemerkt te worden. Mogelijk dat er een gelijkwaardige of grotere hoeveelheid pijn veroorzaakt wordt door het spier- en bottrauma dat met een heupfractuur gepaard gaat. Het ontbreken van een associatie tussen weefseltemperatuurverdeling en pijnverlichting illustreert de hypothese die stelt dat CSCT alleen pijnstillend op de huid werkt en spier- of botweefsel onvoldoende koelt om hier ook pijnverlichting te geven.

Effecten van cryotherapie in een mesenchymaal stamcelmodel

Cryotherapie wordt gebruikt om pijn te verlichten in verschillende musculoskeletale letsels, ondanks dat het effect op de cellen die verantwoordelijk zijn voor botgenezing, onbekend is. Het is wel bekend dat cryotherapie weefselmetabolisme verlaagd[24], maar in het geval van een fractuur of een fixatie van een fractuur is een verhoogd metabolisme gewenst zodat zich een fractuurcallus kan vormen met aansluitend botremodellering, en uiteindelijk botgenezing. Onze resultaten in **hoofdstuk 6**, waaruit bleek dat CSCT in staat is de temperatuur op het botniveau te verlagen in cachectische patiënten, zette ons ertoe om te onderzoeken of

cryotherapie ook nadelige invloed heeft op proliferatie en differentiatie van osteoblast voorlopercellen. Deze cellen zijn verantwoordelijk voor botgenezing en een verminderde functie van deze cellen kan leiden tot een *delayed-union* of *non-union*.

In **hoofdstuk 7** presenteren we de resultaten van de invloed van hypothermie in een MSC-model die gekweekt werden in een hypoxisch milieu. Hiermee geven we inzicht over de totstandkoming van het waargenomen effect van cryotherapie op MSC's. In deze experimenten gebruikten we hypoxemie als surrogaat voor een doorgemaakte fractuur. De combinatie van hypothermie en hypoxemie verlaagde *VEGF-165* gen- en eiwitexpressie, wat een marker is voor vasculogenese. Alhoewel differentiatie en proliferatie van MSC's niet beïnvloed werden, kan het stilleggen van *VEGF-165* gen- en eiwitexpressie implicaties hebben voor de vascularisatie van het fractuurcallus, dit laatste is een later stadium van botgenezing. Deze *in vitro* resultaten impliceren dat behandeling met hypothermie *in vivo* om pijn en inflammatie te verminderen waarschijnlijk geen nadelige gevolgen heeft voor de vroege stadia van botgenezing, maar mogelijk wel voor de latere stadia.

Onze studie is de eerste die de *in vitro* effecten beschrijft van hypothermie in een MSC-model in hypoxische condities, maar meer *in vivo* onderzoek is nodig om harde conclusies te kunnen trekken over het effect van cryotherapie op botgenezing. Bij onze resultaten van **hoofdstuk 6** is op te merken dat CSCT de temperatuur verlaagd naar een gelijkwaardig niveau dat gebruikt is in de MSC-experimenten in **hoofdstuk 7**. Daarom kan de toepassing van CSCT mogelijk nadelige gevolgen hebben voor de latere stadia van botgenezing indien deze voor een langere tijd toegepast wordt.

Conclusies

De studies die besproken worden in dit proefschrift hebben geleid tot de volgende conclusies over de toepassing van CSCT na heupfractuur chirurgie en hypothermie:

- Cryotherapie wordt voornamelijk toegepast na (semi-)electieve chirurgie. Het wordt op een heterogene manier toegepast en blijkt veilig en geeft daarnaast een milde afname van morfinegebruik en bloedverlies;
- Continue-stroming cryocompressietherapie therapie verlaagd postoperatief bloedverlies na THA-OA één dag na de operatie, het verminderd de pijn niet;
- Continue-stroming cryocompressietherapie therapie geeft geen pijnstillende voordelen in de acute herstelperiode na heupfractuurchirurgie, daarnaast vermindert het ook het postoperatief bloedverlies niet;

- Verlaging van de weefseltemperatuur CSCT en pijnbeleving van heupfractuurpatiënten zijn niet gerelateerd. Dit kan suggereren dat cryotherapie-geïnduceerde pijnverlichting voortkomt uit vermindering van huid-gerelateerde pijn, in plaats van spier- of botgerelateerde pijn;
- Hypothermie vermindert *VEGF-165* gen- en eiwitexpressie, maar heeft geen invloed op de differentiatie of apoptose van MSC die gekweekt worden in een hypoxisch milieu. Dit impliceert dat hypothermie-behandeling *in vivo*, toegepast voor pijnverlichting en vermindering van inflammatie, waarschijnlijk de vroege stadia van botgenezing niet schaadt.

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Chapter 10

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About the author

Nicolaas Cornelis (given name Nick) Leegwater was born on March 3rd 1986 in Niedorp, Noord-Holland, The Netherlands, where he spent his childhood and attended the Han Fortmann College in Heerhugowaard. After he graduated in 2005, he started medical school at the VU University in Amsterdam. During first years of his study he joyfully worked for his father's trucking company transporting cargo throughout the European Union during the weekends. Early on in life he was interested in medicine, and especially in broken bones. As a child he scanned through the medical encyclopaedias of his parents looking for pictures of broken bones. During his internships he started conducting research with dr. P.A. Nolte in the Spaarne Gasthuis hospital in Hoofddorp that formed the basis of this thesis. After obtaining his medical degree in 2011 he started as a resident surgery in the VU medical centre while further expanding his PhD research in orthopaedic trauma surgery with dr. Nolte. Beginning 2013 he started his own company "Leegwater Medical Services" through which he worked as an emergency doctor in twelve hospitals all around The Netherlands while simultaneously working on his PhD thesis. He then continued his research in the Spaarne Gasthuis hospital under the supervision of dr. Nolte as PhD researcher. Here he conducted fulltime research for a year and a half after starting in the clinic as orthopaedic surgery resident in the same hospital. In April 2017, he started his orthopaedic surgery training (supervisor dr. M. van Dijk) at the general surgery department at the St. Antonius Hospital in Nieuwegein (supervisor dr. D. Boerma).